

(19)



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11)

EP 0 711 832 A2

(12)

EUROPEAN PATENT APPLICATION

(43) Date of publication:
15.05.1996 Bulletin 1996/20

(51) Int Cl.⁶: **C12N 15/12**, C07K 14/705,
A61K 38/17

(21) Application number: **95308033.0**

(22) Date of filing: **09.11.1995**

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI LU NL PT SE

(30) Priority: **14.11.1994 US 337797**

(71) Applicant: **ELI LILLY AND COMPANY**
Indianapolis, Indiana 46285 (US)

(72) Inventors:
• **Burnett, James Paul, Jr.**
Indianapolis, Indiana 46250 (US)

- **Mayne, Nancy Gail**
Indianapolis, Indiana 46226 (US)
- **Sharp, Robert Leon**
Indianapolis, Indiana 46237 (US)
- **Snyder, Yvonne Marie**
Indianapolis, Indiana 46256 (US)

(74) Representative: **Hudson, Christopher Mark**
Lilly Industries Limited
European Patent Operations
Erl Wood Manor
Windlesham Surrey GU20 6PH (GB)

(54) **Human metabotropic glutamate receptor, m Glu R2**

(57) This invention describes a novel human glutamate receptors, designated mGluR2. This invention also encompasses nucleic acids encoding this receptor,

or a fragment thereof, as well as methods employing this receptor and the nucleic acid compounds.

EP 0 711 832 A2

*m5002
#10*

Description

In the mammalian central nervous system (CNS), the transmission of nerve impulses is controlled by the interaction between a neurotransmitter, that is released by a sending neuron, and a surface receptor on a receiving neuron, which causes excitation of this receiving neuron. L-Glutamate, which is the most abundant neurotransmitter in the CNS, mediates the major excitatory pathway in mammals, and is referred to as an excitatory amino acid (EAA). The receptors that respond to glutamate are called excitatory amino acid receptors (EAA receptors). See Watkins & Evans, Annual Reviews in Pharmacology and Toxicology, 21:165 (1981); Monaghan, Bridges, and Cotman, Annual Reviews in Pharmacology and Toxicology, 29:365 (1989); Watkins, Krogsgaard-Larsen, and Honore, Transactions in Pharmaceutical Science, 11:25 (1990). The excitatory amino acids are of great physiological importance, playing a role in a variety of physiological processes, such as long-term potentiation (learning and memory), the development of synaptic plasticity, motor control, respiration, cardiovascular regulation, and sensory perception.

Excitatory amino acid receptors are classified into two general types. Receptors that are directly coupled to the opening of cation channels in the cell membrane of the neurons are termed "ionotropic." This type of receptor has been subdivided into at least three subtypes, which are defined by the depolarizing actions of the selective agonists N-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and kainic acid (KA).

The second general type of receptor is the G-protein or second messenger-linked "metabotropic" excitatory amino acid receptor. This second type is coupled to multiple second messenger systems that lead to enhanced phosphoinositide hydrolysis, activation of phospholipase D, increases or decreases in cAMP formation, and changes in ion channel function. Schoepp and Conn, Trends in Pharmacological science, 14:13 (1993). Both types of receptors appear not only to mediate normal synaptic transmission along excitatory pathways, but also participate in the modification of synaptic connections during development and throughout life. Schoepp, Bockaert, and Sladeczek, Trends in Pharmacological science, 11:508 (1990); McDonald and Johnson, Brain Research reviews, 15:41 (1990).

The excessive or inappropriate stimulation of excitatory amino acid receptors leads to neuronal cell damage or loss by way of a mechanism known as excitotoxicity. This process has been suggested to mediate neuronal degeneration in a variety of conditions. The medical consequences of such neuronal degeneration makes the abatement of these degenerative neurological processes an important therapeutic goal.

The metabotropic glutamate receptors are a highly heterogeneous family of glutamate receptors that are linked to multiple second-messenger pathways. These receptors function to modulate the presynaptic release of glutamate, and the postsynaptic sensitivity of the neuronal cell to glutamate excitation. Agonists and antagonists of these receptors may be useful for the treatment of acute and chronic neurodegenerative conditions, and as antipsychotic, anticonvulsant, analgesic, anxiolytic, antidepressant, and anti-emetic agents.

The present invention provides an additional human excitatory amino acid receptor, designated mGluR2, to those previously known. The characterization and treatment of physiological disorders is hereby furthered.

This invention provides an isolated amino acid compound useful as a human metabotropic glutamate receptor, said compound comprising the amino acid sequence

EP 0 711 832 A2

	Met	Gly	Ser	Leu	Leu	Ala	Leu	Leu	Ala	Leu	Leu	Pro	Leu	Trp	Gly	Ala	
	1				5					10					15		
5	Val	Ala	Glu	Gly	Pro	Ala	Lys	Lys	Val	Leu	Thr	Leu	Glu	Gly	Asp	Leu	
				20					25					30			
	Val	Leu	Gly	Gly	Leu	Phe	Pro	Val	His	Gln	Lys	Gly	Gly	Pro	Ala	Glu	
10			35					40					45				
	Asp	Cys	Gly	Pro	Val	Asn	Glu	His	Arg	Gly	Ile	Gln	Arg	Leu	Glu	Ala	
		50					55					60					
15	Met	Leu	Phe	Ala	Leu	Asp	Arg	Ile	Asn	Arg	Asp	Pro	His	Leu	Leu	Pro	
	65					70					75						
	Gly	Val	Arg	Leu	Gly	Ala	His	Ile	Leu	Asp	Ser	Cys	Ser	Lys	Asp	Thr	
					85					90					95		
20	His	Ala	Leu	Glu	Gln	Ala	Leu	Asp	Phe	Val	Arg	Ala	Ser	Leu	Ser	Arg	
				100					105					110			
	Gly	Ala	Asp	Gly	Ser	Arg	His	Ile	Cys	Pro	Asp	Gly	Ser	Tyr	Ala	Thr	
25			115					120					125				
	His	Gly	Asp	Ala	Pro	Thr	Ala	Ile	Thr	Gly	Val	Ile	Gly	Gly	Ser	Tyr	
		130					135					140					
30	Ser	Asp	Val	Ser	Ile	Gln	Val	Ala	Asn	Leu	Leu	Arg	Leu	Phe	Gln	Ile	
	145					150					155				160		
	Pro	Gln	Ile	Ser	Tyr	Ala	Ser	Thr	Ser	Ala	Lys	Leu	Ser	Asp	Lys	Ser	
				165						170					175		
35	Arg	Tyr	Asp	Tyr	Phe	Ala	Arg	Thr	Val	Pro	Pro	Asp	Phe	Phe	Gln	Ala	
				180					185					190			
	Lys	Ala	Met	Ala	Glu	Ile	Leu	Arg	Phe	Phe	Asn	Trp	Thr	Tyr	Val	Ser	
40			195					200					205				
	Thr	Val	Ala	Ser	Glu	Gly	Asp	Tyr	Gly	Glu	Thr	Gly	Ile	Glu	Ala	Phe	
		210					215					220					
45	Glu	Leu	Glu	Ala	Arg	Ala	Arg	Asn	Ile	Cys	Val	Ala	Thr	Ser	Glu	Lys	
	225					230					235					240	
	Val	Gly	Arg	Ala	Met	Ser	Arg	Ala	Ala	Phe	Glu	Gly	Val	Val	Arg	Ala	
					245					250					255		
50																	
55																	

EP 0 711 832 A2

	Leu	Leu	Gln	Lys	Pro	Ser	Ala	Arg	Val	Ala	Val	Leu	Phe	Thr	Arg	Ser	
				260					265						270		
5	Glu	Asp	Ala	Arg	Glu	Leu	Leu	Ala	Ala	Ser	Gln	Arg	Leu	Asn	Ala	Ser	
			275					280					285				
	Phe	Thr	Trp	Val	Ala	Ser	Asp	Gly	Trp	Gly	Ala	Leu	Glu	Ser	Val	Val	
10			290				295					300					
	Ala	Gly	Ser	Glu	Gly	Ala	Ala	Glu	Gly	Ala	Ile	Thr	Ile	Glu	Leu	Ala	
	305					310					315					320	
	Ser	Tyr	Pro	Ile	Ser	Asp	Phe	Ala	Ser	Tyr	Phe	Gln	Ser	Leu	Asp	Pro	
15				325						330					335		
	Trp	Asn	Asn	Ser	Arg	Asn	Pro	Trp	Phe	Arg	Glu	Phe	Trp	Glu	Gln	Arg	
				340					345					350			
20	Phe	Arg	Cys	Ser	Phe	Arg	Gln	Arg	Asp	Cys	Ala	Ala	His	Ser	Leu	Arg	
			355					360					365				
	Ala	Val	Pro	Phe	Glu	Gln	Glu	Ser	Lys	Ile	Met	Phe	Val	Val	Asn	Ala	
25			370				375					380					
	Val	Tyr	Ala	Met	Ala	His	Ala	Leu	His	Asn	Met	His	Arg	Ala	Leu	Cys	
	385				390					395						400	
	Pro	Asn	Thr	Thr	Arg	Leu	Cys	Asp	Ala	Met	Arg	Pro	Val	Asn	Gly	Arg	
30					405					410					415		
	Arg	Leu	Tyr	Lys	Asp	Phe	Val	Leu	Asn	Val	Lys	Phe	Asp	Ala	Pro	Phe	
				420					425					430			
35	Arg	Pro	Ala	Asp	Thr	His	Asn	Glu	Val	Arg	Phe	Asp	Arg	Phe	Gly	Asp	
			435					440					445				
	Gly	Ile	Gly	Arg	Tyr	Asn	Ile	Phe	Thr	Tyr	Leu	Arg	Ala	Gly	Ser	Gly	
40			450				455					460					
	Arg	Tyr	Arg	Tyr	Gln	Lys	Val	Gly	Tyr	Trp	Ala	Glu	Gly	Leu	Thr	Leu	
	465				470					475						480	
	Asp	Thr	Ser	Leu	Ile	Pro	Trp	Ala	Ser	Pro	Ser	Ala	Gly	Pro	Leu	Pro	
45					485					490					495		
	Ala	Ser	Arg	Cys	Ser	Glu	Pro	Cys	Leu	Gln	Asn	Glu	Val	Lys	Ser	Val	
				500					505					510			
50	Gln	Pro	Gly	Glu	Val	Cys	Cys	Trp	Leu	Cys	Ile	Pro	Cys	Gln	Pro	Tyr	
			515					520					525				
	Glu	Tyr	Arg	Leu	Asp	Glu	Phe	Thr	Cys	Ala	Asp	Cys	Gly	Leu	Gly	Tyr	
55			530				535					540					

EP 0 711 832 A2

Trp Pro Asn Ala Ser Leu Thr Gly Cys Phe Glu Leu Pro Gln Glu Tyr
 545 550 555 560
 5
 Ile Arg Trp Gly Asp Ala Trp Ala Val Gly Pro Val Thr Ile Ala Cys
 565 570 575
 Leu Gly Ala Leu Ala Thr Leu Phe Val Leu Gly Val Phe Val Arg His
 580 585 590
 10
 Asn Ala Thr Pro Val Val Lys Ala Ser Gly Arg Glu Leu Cys Tyr Ile
 595 600 605
 Leu Leu Gly Gly Val Phe Leu Cys Tyr Cys Met Thr Phe Ile Phe Ile
 610 615 620
 15
 Ala Lys Pro Ser Thr Gly Val Cys Ala Leu Arg Arg Leu Gly Val Gly
 625 630 635 640
 20
 Thr Ala Phe Ser Val Cys Tyr Ser Ala Leu Leu Thr Lys Thr Asn Arg
 645 650 655
 Ile Ala Arg Ile Phe Gly Gly Ala Arg Glu Gly Ala Gln Arg Pro Arg
 660 665 670
 25
 Phe Ile Ser Pro Ala Ser Gln Val Ala Ile Cys Leu Ala Leu Ile Ser
 675 680 685
 Gly Gln Leu Leu Ile Val Val Ala Trp Leu Val Val Glu Ala Pro Gly
 690 695 700
 30
 Thr Gly Lys Glu Thr Ala Pro Glu Arg Arg Glu Val Val Thr Leu Arg
 705 710 715 720
 35
 Cys Asn His Arg Asp Ala Ser Met Leu Gly Ser Leu Ala Tyr Asn Val
 725 730 735
 Leu Leu Ile Ala Leu Cys Thr Leu Tyr Ala Phe Lys Thr Arg Lys Cys
 740 745 750
 40
 Pro Glu Asn Phe Asn Glu Ala Lys Phe Ile Gly Phe Thr Met Tyr Thr
 755 760 765
 Thr Cys Ile Ile Trp Leu Ala Phe Leu Pro Ile Phe Tyr Val Thr Ser
 770 775 780
 45
 Ser Asp Tyr Arg Val Gln Thr Thr Thr Met Cys Val Ser Val Ser Leu
 785 790 795 800
 50
 Ser Gly Ser Val Val Leu Gly Cys Leu Phe Ala Pro Lys Leu His Ile
 805 810 815
 55

Ile Leu Phe Gln Pro Gln Lys Asn Val Val Ser His Arg Ala Pro Thr
820 825 830

5 Ser Arg Phe Gly Ser Ala Ala Ala Arg Ala Ser Ser Ser Leu Gly Gln
835 840 845

Gly Ser Gly Ser Gln Phe Val Pro Thr Val Val Cys Asn Gly Arg Glu Val
850 855 860

10 Val Asp Ser Thr Thr Ser Ser Leu
865 870

hereinafter designated as SEQ ID NO:2.

15 The invention also provides an isolated nucleic acid compound that comprises a nucleic acid sequence which encodes for the amino acid compounds provided. Particularly this invention provides the isolated nucleic acid compound having the sequence

20	ATG GGA TCG CTG CTT GCG CTC CTG GCA CTG CTG CCG CTC TGG GGT GCT Met Gly Ser Leu Leu Ala Leu Leu Ala Leu Leu Pro Leu Trp Gly Ala 1 5 10 15	48
25	GTG GCT GAG GGC CCA GCC AAG AAG GTG CTG ACC CTG GAG GGA GAC TTG Val Ala Glu Gly Pro Ala Lys Lys Val Leu Thr Leu Glu Gly Asp Leu 20 25 30	96
30	GTG CTG GGT GGG CTG TTC CCA GTG CAC CAG AAG GGC GGC CCA GCA GAG Val Leu Gly Gly Leu Phe Pro Val His Gln Lys Gly Gly Pro Ala Glu 35 40 45	144
35	GAC TGT GGT CCT GTC AAT GAG CAC CGT GGC ATC CAG CCG CTG GAG GCC Asp Cys Gly Pro Val Asn Glu His Arg Gly Ile Gln Arg Leu Glu Ala 50 55 60	192
40	ATG CTT TTT GCA CTG GAC CGC ATC AAC CGT GAC CCG CAC CTG CTG CCT Met Leu Phe Ala Leu Asp Arg Ile Asn Arg Asp Pro His Leu Leu Pro 65 70 75 80	240
45	GGC GTG CGC CTG GGT GCA CAC ATC CTC GAC AGT TGC TCC AAG GAC ACA Gly Val Arg Leu Gly Ala His Ile Leu Asp Ser Cys Ser Lys Asp Thr 85 90 95	288
50	CAT GCG CTG GAG CAG GCA CTG GAC TTT GTG CGT GGC TCA CTC AGC CGT His Ala Leu Glu Gln Ala Leu Asp Phe Val Arg Ala Ser Leu Ser Arg 100 105 110	336
55	GGT GCT GAT GGC TCA CGC CAC ATC TGC CCC GAC GGC TGT TAT GCG ACC Gly Ala Asp Gly Ser Arg His Ile Cys Pro Asp Gly Ser Tyr Ala Thr 115 120 125	384

EP 0 711 832 A2

	CAT	GGT	GAT	GCT	CCC	ACT	GCC	ATC	ACT	GGT	GTT	ATT	GGC	GGT	TCC	TAC	432
	His	Gly	Asp	Ala	Pro	Thr	Ala	Ile	Thr	Gly	Val	Ile	Gly	Gly	Ser	Tyr	
		130					135					140					
5	AST	GAT	GTC	TCC	ATC	CAG	GTG	GCC	AAC	CTC	TTG	AGG	CTA	TTT	CAG	ATC	480
	Ser	Asp	Val	Ser	Ile	Gln	Val	Ala	Asn	Leu	Leu	Arg	Leu	Phe	Gln	Ile	
	145					150					155					160	
10	CCA	CAG	ATT	AGC	TAC	GCC	TCT	ACC	AGT	GCC	AAG	CTG	AGT	GAC	AAG	TCC	528
	Pro	Gln	Ile	Ser	Tyr	Ala	Ser	Thr	Ser	Ala	Lys	Leu	Ser	Asp	Lys	Ser	
					165					170					175		
15	CGC	TAT	GAC	TAC	TTT	GCC	CGC	ACA	GTG	CCT	CCT	GAC	TTC	TTC	CAA	GCC	576
	Arg	Tyr	Asp	Tyr	Phe	Ala	Arg	Thr	Val	Pro	Pro	Asp	Phe	Phe	Gln	Ala	
				180					185					190			
20	AAG	GCC	ATG	GCT	GAG	ATT	CTC	CGC	TTC	TTC	AAC	TGG	ACC	TAT	GTG	TCC	624
	Lys	Ala	Met	Ala	Glu	Ile	Leu	Arg	Phe	Phe	Asn	Trp	Thr	Tyr	Val	Ser	
		195					200					205					
25	ACT	GTG	GCG	TCT	GAG	GGC	GAC	TAT	GGC	GAG	ACA	GGC	ATT	GAG	GCC	TTT	672
	Thr	Val	Ala	Ser	Glu	Gly	Asp	Tyr	Gly	Glu	Thr	Gly	Ile	Glu	Ala	Phe	
		210				215						220					
30	GAG	CTA	GAG	GCT	CGT	GCC	CGC	AAC	ATC	TGT	GTG	GCC	ACC	TCG	GAG	AAA	720
	Glu	Leu	Glu	Ala	Arg	Ala	Arg	Asn	Ile	Cys	Val	Ala	Thr	Ser	Glu	Lys	
	225					230					235				240		
35	GTG	GGC	CGT	GCC	ATG	AGC	CGC	GCG	GCC	TTT	GAG	GGT	GTG	GTG	CGA	GCC	768
	Val	Gly	Arg	Ala	Met	Ser	Arg	Ala	Ala	Phe	Glu	Gly	Val	Val	Arg	Ala	
					245					250					255		
40	CTG	CTG	CAG	AAG	CCC	AGT	GCC	CGC	GTG	GCT	GTC	CTG	TTC	ACC	CGT	TCT	816
	Leu	Leu	Gln	Lys	Pro	Ser	Ala	Arg	Val	Ala	Val	Leu	Phe	Thr	Arg	Ser	
				260					265					270			
45	GAG	GAT	GCC	CGG	GAG	CTG	CTT	GCT	GCC	AGC	CAG	CGC	CTC	AAT	GCC	AGC	864
	Glu	Asp	Ala	Arg	Glu	Leu	Leu	Ala	Ala	Ser	Gln	Arg	Leu	Asn	Ala	Ser	
		275						280					285				
50	TTC	ACC	TGG	GTG	GCC	AGT	GAT	GGT	TGG	GGG	GCC	CTG	GAG	AGT	GTG	GTG	912
	Phe	Thr	Trp	Val	Ala	Ser	Asp	Gly	Trp	Gly	Ala	Leu	Glu	Ser	Val	Val	
		290					295				300						
55	GCA	GCC	AGT	GAG	GGG	GCT	GCT	GAG	GGT	GCT	ATC	ACC	ATC	GAG	CTG	GCC	960
	Ala	Gly	Ser	Glu	Gly	Ala	Ala	Glu	Gly	Ala	Ile	Thr	Ile	Glu	Leu	Ala	
	305					310					315				320		
60	TCC	TAC	CCC	ATC	AGT	GAC	TTT	GCC	TCC	TAC	TTC	CAG	AGC	CTG	GAC	CCT	1008
	Ser	Tyr	Pro	Ile	Ser	Asp	Phe	Ala	Ser	Tyr	Phe	Gln	Ser	Leu	Asp	Pro	
					325					330					335		

EP 0 711 832 A2

	TGG	AAC	AAC	AGC	CGG	AAC	CCC	TGG	TTC	CGT	GAA	TTC	TGG	GAG	CAG	AGG	1056
	Trp	Asn	Asn	Ser	Arg	Asn	Pro	Trp	Phe	Arg	Glu	Phe	Trp	Gln	Gln	Arg	
5				340					345					350			
	TTC	CGC	TGC	AGC	TTC	CGG	CAG	CGA	GAC	TGC	GCA	GCC	CAC	TCT	CTC	CGG	1104
	Phe	Arg	Cys	Ser	Phe	Arg	Gln	Arg	Asp	Cys	Ala	Ala	His	Ser	Leu	Arg	
			355					360					365				
10	GCT	GTG	CCC	TTT	GAG	CAG	GAG	TCC	AAG	ATC	ATG	TTT	GTG	GTC	AAT	GCA	1152
	Ala	Val	Pro	Phe	Glu	Gln	Glu	Ser	Lys	Ile	Met	Phe	Val	Val	Asn	Ala	
		370					375					380					
15	GTG	TAC	GCC	ATG	GCC	CAT	GCG	CTC	CAC	AAC	ATG	CAC	CGT	GCC	CTC	TGC	1200
	Val	Tyr	Ala	Met	Ala	His	Ala	Leu	His	Asn	Met	His	Arg	Ala	Leu	Cys	
	385					390				395					400		
20	CCC	AAC	ACC	ACC	CGG	CTC	TGT	GAC	GCG	ATG	CGG	CCA	GTT	AAC	GGG	CGC	1248
	Pro	Asn	Thr	Thr	Arg	Leu	Cys	Asp	Ala	Met	Arg	Pro	Val	Asn	Gly	Arg	
					405				410						415		
25	CGC	CTC	TAC	AAG	GAC	TTT	GTG	CTC	AAC	ATC	AAG	TTT	GAT	GCC	CCC	TTT	1296
	Arg	Leu	Tyr	Lys	Asp	Phe	Val	Leu	Asn	Val	Lys	Phe	Asp	Ala	Pro	Phe	
			420						425					430			
30	CGC	CCA	GCT	GAC	ACC	CAC	AAT	GAG	GTC	CGC	TTT	GAC	CGC	TTT	GGT	GAT	1344
	Arg	Pro	Ala	Asp	Thr	His	Asn	Glu	Val	Arg	Phe	Asp	Arg	Phe	Gly	Asp	
		435						440					445				
35	GGT	ATT	GGC	CGC	TAC	AAC	ATC	TTC	ACC	TAT	CTG	CGT	GCA	GGC	AGT	GGG	1392
	Gly	Ile	Gly	Arg	Tyr	Asn	Ile	Phe	Thr	Tyr	Leu	Arg	Ala	Gly	Ser	Gly	
	450					455					460						
40	CGC	TAT	CGC	TAC	CAG	AAG	GTG	GGC	TAC	TGG	GCA	GAA	GGC	TTG	ACT	CTG	1440
	Arg	Tyr	Arg	Tyr	Gln	Lys	Val	Gly	Tyr	Trp	Ala	Glu	Gly	Leu	Thr	Leu	
	465				470				475					480			
45	GAC	ACC	AGC	CTC	ATC	CCA	TGG	GCC	TCA	CCC	TCA	GCC	GGC	CCC	CTG	CCC	1488
	Asp	Thr	Ser	Leu	Ile	Pro	Trp	Ala	Ser	Pro	Ser	Ala	Gly	Pro	Leu	Pro	
				485					490					495			
50	GCC	TCT	CGC	TGC	AGT	GAG	CCC	TGC	CTC	CAG	AAT	GAG	GTG	AAG	AGT	GTG	1536
	Ala	Ser	Arg	Cys	Ser	Glu	Pro	Cys	Leu	Gln	Asn	Glu	Val	Lys	Ser	Val	
			500					505					510				
55	CAG	CCG	GGC	GAA	GTC	TGC	TGC	TGG	CTC	TGC	ATT	CCG	TGC	CAG	CCC	TAT	1584
	Gln	Pro	Gly	Glu	Val	Cys	Cys	Trp	Leu	Cys	Ile	Pro	Cys	Gln	Pro	Tyr	
			515					520					525				
60	GAG	TAC	CGA	TTG	GAC	GAA	TTC	ACT	TGC	GCT	GAT	TGT	GGC	CTG	GGC	TAC	1632
	Glu	Tyr	Arg	Leu	Asp	Glu	Phe	Thr	Cys	Ala	Asp	Cys	Gly	Leu	Gly	Tyr	
		530					535					540					

EP 0 711 832 A2

5	TGG CCC AAT GCC AGC CTG ACT GGC TGC TTC GAA CTG CCC CAG GAG TAC Trp Pro Asn Ala Ser Leu Thr Gly Cys Phe Glu Leu Pro Gln Glu Tyr 545 550 555 560	1680
10	ATC CGC TGG GGC GAT GCC TGG GCT GTG GGA CCT GTC ACC ATC GCC TGC Ile Arg Trp Gly Asp Ala Trp Ala Val Gly Pro Val Thr Ile Ala Cys 565 570 575	1728
15	CTC GGT GCC CTG GCC ACC CTC TTT GTG CTG GGT GTC TTT GTG CGG CAC Leu Gly Ala Leu Ala Thr Leu Phe Val Leu Gly Val Phe Val Arg His 580 585 590	1776
20	AAT GCC ACA CCA GTG GTC AAG GCC TCA GGT CGG GAG CTC TGC TAC ATC Asn Ala Thr Pro Val Val Lys Ala Ser Gly Arg Glu Leu Cys Tyr Ile 595 600 605	1824
25	CTG CTG GGT GGT GTC TTC CTC TGC TAC TGC ATG ACC TTC ATC TTC ATT Leu Leu Gly Gly Val Phe Leu Cys Tyr Cys Met Thr Phe Ile Phe Ile 610 615 620	1872
30	GCC AAG CCA TCC ACG GGA GTG TGT GCC TTA CGG CGT CTT GGG GTG GGC Ala Lys Pro Ser Thr Gly Val Cys Ala Leu Arg Arg Leu Gly Val Gly 625 630 635 640	1920
35	ACT GCC TTC TCT GTC TGC TAC TCA GCC CTG CTC ACC AAG ACC AAC CGC Thr Ala Phe Ser Val Cys Tyr Ser Ala Leu Leu Thr Lys Thr Asn Arg 645 650 655	1968
40	ATT GCA CGC ATC TTC GGT GGG GCC CGG GAG GGT GCC CAG CGG CCA CGC Ile Ala Arg Ile Phe Gly Gly Ala Arg Glu Gly Ala Gln Arg Pro Arg 660 665 670	2016
45	TTC ATC AGT CCT GCC TCA CAG GTG GCC ATC TGC CTG GCA CTT ATC TCG Phe Ile Ser Pro Ala Ser Gln Val Ala Ile Cys Leu Ala Leu Ile Ser 675 680 685	2064
50	GGC CAG CTG CTC ATC GTG GTC GCC TGG CTG GTG GTG GAG GCA CCG GGC Gly Gln Leu Leu Ile Val Val Ala Trp Leu Val Val Glu Ala Pro Gly 690 695 700	2112
55	ACA GGC AAG GAG ACA GCC CCC GAA CGG CGG GAG GTG GTG ACA CTG CGC Thr Gly Lys Glu Thr Ala Pro Glu Arg Arg Glu Val Val Thr Leu Arg 705 710 715 720	2160
60	TGC AAC CAC CGC GAT GCA AGT ATG TTG GGC TCG CTG GCC TAC AAT GTG Cys Asn His Arg Asp Ala Ser Met Leu Gly Ser Leu Ala Tyr Asn Val 725 730 735	2208
65	CTC CTC ATC GCG CTC TGC ACG CTT TAT GCC TTC AAG ACT CGC AAG TGC Leu Leu Ile Ala Leu Cys Thr Leu Tyr Ala Phe Lys Thr Arg Lys Cys 740 745 750	2256
70	CCC GAA AAC TTC AAC GAG GCC AAG TTC ATT GGC TTC ACC ATG TAC ACC	2304

	Pro	Glu	Asn	Phe	Asn	Glu	Ala	Lys	Phe	Ile	Gly	Phe	Thr	Met	Tyr	Thr	
			755					760					765				
5	ACC	TGC	ATC	ATC	TGG	CTG	GCA	TTC	TTG	CCC	ATC	TTC	TAT	GTC	ACC	TCC	2352
	Thr	Cys	Ile	Ile	Trp	Leu	Ala	Phe	Leu	Pro	Ile	Phe	Tyr	Val	Thr	Ser	
		770					775					780					
10	AGT	GAC	TAC	CGG	GTA	CAG	ACC	ACC	ACC	ATG	TGC	GTG	TCA	GTC	AGC	CTC	2400
	Ser	Asp	Tyr	Arg	Val	Gln	Thr	Thr	Thr	Met	Cys	Val	Ser	Val	Ser	Leu	
		785				790					795					800	
15	AGC	GGC	TCC	GTG	GTG	CTT	GGC	TGC	CTC	TTT	GCG	CCC	AAG	CTG	CAC	ATC	2448
	Ser	Gly	Ser	Val	Val	Leu	Gly	Cys	Leu	Phe	Ala	Pro	Lys	Leu	His	Ile	
					805					810					815		
20	ATC	CTC	TTC	CAG	CCG	CAG	AAG	AAC	GTG	GTT	AGC	CAC	CGG	GCA	CCC	ACC	2496
	Ile	Leu	Phe	Gln	Pro	Gln	Lys	Asn	Val	Val	Ser	His	Arg	Ala	Pro	Thr	
				820					825					830			
25	AGC	CGC	TTT	GSC	AGT	GCT	GCT	GCC	AGG	GCC	AGC	TCC	AGC	CTT	GGC	CAA	2544
	Ser	Arg	Phe	Gly	Ser	Ala	Ala	Ala	Arg	Ala	Ser	Ser	Ser	Leu	Gly	Gln	
			835					840					845				
30	GGG	TCT	GGC	TCC	CAG	TTT	GTC	CCC	ACT	GTT	TGC	AAT	GGC	CGT	GAG	GTG	2592
	Gly	Ser	Gly	Ser	Gln	Phe	Val	Pro	Thr	Val	Cys	Asn	Gly	Arg	Glu	Val	
			850				855					860					
35	GTG	GAC	TCG	ACA	ACG	TCA	TCG	CTT	TGA								2619
	Val	Asp	Ser	Thr	Thr	Ser	Ser	Leu									
							865		870								

which is hereinafter designated as SEQ ID NO:1.

This invention also provides recombinant nucleic acid vectors comprising nucleic acids encoding SEQ ID NO:2.

This invention also encompasses recombinant DNA vectors which comprise the isolated DNA sequence which is SEQ ID NO:1.

The present invention also provides assays for determining the efficacy and adverse reaction profile of agents useful in the treatment or prevention of disorders associated with an excess or deficiency in the amount of glutamate present.

Brief Description of the Figures

Figure 1 is a restriction and function map of the plasmid pRS133. The arc having the wider line indicates that portion of the plasmid which corresponds to SEQ ID NO:1, *infra*. The arrow delineates that region of the insert which encodes the protein of SEQ ID NO:2 with the direction of the arrow indicating the natural order of transcription from the 5' end to the 3' end. The designation "ORI" refers to the plasmid origin of replication. The designation "f1 ori" refers to phage f1-derived origin of replication. The designation "amp^r" refers to the gene encoding ampicillin resistance. The designations "T7" and "T3" refer to the T7 promoter and the T3 promoter, respectively.

Figure 2 is a restriction and function map of the plasmid pRS138. The arc having the wider line indicates that portion of the plasmid which corresponds to SEQ ID NO:1, *infra*. The arrow delineates that region of the insert which encodes the protein of SEQ ID NO:2 with the direction of the arrow indicating the natural order of transcription from the 5' end to the 3' end. The designation "EP" refers to the SV40 early promoter. "Enh" refers to the BK enhancer in tandem with the adenovirus late promoter. "Poly A" refers to the SV40 polyadenylation site. The term "3' splice" refers to the 3' splice site derived from SV40. The designation "ori" refers to a pBR322-derived origin of replication. The designation "amp^r" refers to the gene encoding ampicillin resistance. The designation "hyg^r" refers to the gene encoding hygromycin resistance.

The terms and abbreviations used in this document have their normal meanings unless otherwise designated. For example "°C" refers to degrees Celsius; "N" refers to normal or normality; "mmol" refers to millimole or millimoles; "g"

refers to gram or grams; "ml" means milliliter or milliliters; "M" refers to molar or molarity; "µg" refers to microgram or micrograms; and "µl" refers to microliter or microliters.

All nucleic acid sequences, unless otherwise designated, are written in the direction from the 5' end to the 3' end, frequently referred to as "5' to 3'".

5 All amino acid or protein sequences, unless otherwise designated, are written commencing with the amino terminus ("N-terminus") and concluding with the carboxy terminus ("C-terminus").

"Base pair" or "bp" as used herein refers to DNA or RNA. The abbreviations A,C,G, and T correspond to the 5'-monophosphate forms of the deoxyribonucleosides (deoxy)adenine, (deoxy)cytidine, (deoxy) guanine, and (deoxy) thymine, respectively, when they occur in DNA molecules. The abbreviations U,C,G, and T correspond to the 5'-mono-phosphate forms of the ribonucleosides uracil, cytidine, guanine, and thymine, respectively when they occur in RNA molecules. In double stranded DNA, base pair may refer to a partnership of A with T or C with G. In a DNA/RNA, heteroduplex base pair may refer to a partnership of A with U or C with G. (See the definition of "complementary", *infra*.)

10 The terms "digestion" or "restriction" of DNA refers to the catalytic cleavage of the Dig, with a restriction enzyme that acts only at certain sequences in the DNA ("sequence-specific endonucleases"). The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors, and other requirements were used as would be known to one of ordinary skill in the art. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer or can be readily found in the literature.

15 "Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (T. Maniatis, *et al.*, *supra*, p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with a DNA ligase, such as T4 DNA ligase.

20 The term "plasmid" refers to an extrachromosomal (usually) self-replicating genetic element. Plasmids are generally designated by a lower case "p" preceded and/or followed by letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accordance with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

25 The term "reading frame" means the nucleotide sequence from which translation occurs "read" in triplets by the translational apparatus of transfer RNA (tRNA) and ribosomes and associated factors, each triplet corresponding to a particular amino acid. A base pair insertion or deletion (termed a frameshift mutation) may result in two different proteins being coded for by the same DNA segment. To insure against this, the triplet codons corresponding to the desired polypeptide must be aligned in multiples of three from the initiation codon, i.e. the correct "reading frame" being main-
30 tained.

"Recombinant DNA cloning vector" as used herein refers to any autonomously replicating agent, including, but not limited to, plasmids and phages, comprising a DNA molecule to which one or more additional DNA segments can or have been added.

35 The term "recombinant DNA expression vector" as used herein refers to any recombinant DNA cloning vector in which a promoter to control transcription of the inserted DNA has been incorporated.

The term "expression vector system" as used herein refers to a recombinant expression vector in combination with one or more trans-acting factors that specifically influence transcription, stability, or replication of the recombinant DNA expression vector. The trans-acting factor may be expressed from a co-transfected plasmid, virus, or other ex-
40 trachromosomal element, or may be expressed from a gene integrated within the chromosome.

"Transcription" as used herein refers to the process whereby information contained in a nucleotide sequence of DNA is transferred to a complementary RNA sequence.

45 The term "transfection" as used herein refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, calcium phosphate co-precipitation, and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

50 The term "transformation" as used herein means the introduction of DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integration. Methods of transforming bacterial and eukaryotic hosts are well known in the art, many of which methods, such as nuclear injection, protoplast fusion or by calcium treatment using calcium chloride are summarized in J. Sambrook, *et al.*, "Molecular Cloning: A Laboratory Manual" (1989).

The term "translation" as used herein refers to the process whereby the genetic information of messenger RNA is used to specify and direct the synthesis of a polypeptide chain.

55 The term "vector" as used herein refers to a nucleic acid compound used for the transformation of cells in gene manipulation bearing polynucleotide sequences corresponding to appropriate protein molecules which when combined with appropriate control sequences confer specific properties on the host cell to be transformed. Plasmids, viruses, and bacteriophage are suitable vectors. Artificial vectors are constructed by cutting and joining DNA molecules from different sources using restriction enzymes and ligases. The term "vector" as used herein includes Recombinant DNA

cloning vectors and Recombinant DNA expression vectors.

The terms "complementary" or "complementarity" as used herein refers to pair of bases, purines and pyrimidines, that associate through hydrogen bonding in double stranded nucleic acid. The following base pairs are complementary: guanine and cytosine; adenine and thymine; and adenine and uracil.

The term "hybridization" as used herein refers to a process in which a strand of nucleic acid joins with a complementary strand through base pairing. The conditions employed in the hybridization of two non-identical, but very similar, complementary nucleic acids varies with the degree of complementarity of the two strands and the length of the strands. Such techniques and conditions are well known to practitioners in this field.

"Isolated amino acid sequence" refers to any amino acid sequence, however constructed or synthesized, which is locationally distinct from the naturally occurring sequence.

"Isolated DNA compound" refers to any DNA sequence, however constructed or synthesized, which is locationally distinct from its natural location in genomic DNA.

"Isolated nucleic acid compound" refers to any RNA or DNA sequence, however constructed or synthesized, which is locationally distinct from its natural location.

A "primer" is a nucleic acid fragment which functions as an initiating substrate for enzymatic or synthetic elongation.

The term "promoter" refers to a DNA sequence which directs transcription of DNA to RNA.

A "probe" as used herein is a nucleic acid compound or a fragment thereof which hybridizes with a nucleic acid compound which encodes either the entire sequence SEQ ID NO:2, a sequence complementary to SEQ ID NO:2, or a part thereof.

The term "stringency" refers to a set of hybridization conditions which may be varied in order to vary the degree of nucleic acid affinity for other nucleic acid. (See the definition of "hybridization", *supra*.)

The term "antigenically distinct" as used herein refers to a situation in which antibodies raised against an epitope of the proteins of the present invention, or a fragment thereof, may be used to differentiate between the proteins of the present invention and other glutamate receptor subtypes. This term may also be employed in the sense that such antibodies may be used to differentiate between the human mGluR2 receptor protein and analogous proteins derived from other species.

The term "PCR" as used herein refers to the widely-known polymerase chain reaction employing a thermally-stable polymerase.

This invention provides the protein of SEQ ID NO:2, a human metabotropic glutamate receptor, designated as a mGluR2 receptor using the nomenclature system described in D.D. Schoepp, "Glutamate receptors", Handbook of Receptors and Channels, Chapter 13 (S.J. Peroutka, ed., CRC Press, 1984). Based on the rat cognate of this receptor, the mGluR2 receptor is believed to be found in a large number of tissues throughout the body, including many regions of the brain such as the Golgi cells of the cerebellum. H. Ohishi, *et al.*, Neuroscience, 53:1009-1018 (1993). Marked expression of mGluR2 messenger RNA is also found in the mitral cells of the accessory olfactory bulb, neurons in the external part of the anterior olfactory nucleus, and pyramidal neurons in the entorhinal and parasubicular cortical regions. The granule cells of the accessory olfactory bulb, and many pyramidal and non-pyramidal neurons in the neocortical, cingulate, retrosplenial, and subicular cortices, were moderately labeled. All of the granule cells in the dentate gyrus were also labeled moderately.

In the basal forebrain regions, moderately labeled neurons are distributed in the triangular septal nucleus, in the lateral, basolateral, and basomedial amygdaloid nuclei, and in the medial mammillary nucleus. Weakly labeled neurons are sparsely scattered in the striatum, globus pallidus, ventral pallidum, and claustrum. The subthalamic nucleus is also labeled weakly. In the thalamus, moderately labeled are distributed in the anterodorsal, antromedial, ventromedial, intralaminar, and midline nuclei; the ventrolateral part of the anteroventral nucleus and the rostral pole of the ventrolateral nucleus also contain moderately labeled neurons.

This receptor is believed to potentiate central nervous system responses and is, therefore, an important target for pharmaceutical purposes.

Skilled artisans will recognize that the proteins of the present invention can be synthesized by a number of different methods. All of the amino acid compounds of the invention can be made by chemical methods well known in the art, including solid phase peptide synthesis, or recombinant methods. Both methods are described in U.S. Patent 4,617,149, herein incorporated by reference.

The principles of solid phase chemical synthesis of polypeptides are well known in the art and may be found in general texts in the area. See, e.g., H. Dugas and C. Penney, Bioorganic Chemistry (1981) Springer-Verlag, New York, pgs. 54-92. For examples, peptides may be synthesized by solid-phase methodology utilizing an Applied Biosystems 430A peptide synthesizer (commercially available from Applied Biosystems, Foster City California) and synthesis cycles supplied by Applied Biosystems. Protected amino acids, such as t-butoxycarbonyl-protected amino acids, and other reagents are commercially available from many chemical supply houses.

Sequential t-butoxycarbonyl chemistry using double couple protocols are applied to the starting p-methyl benzhydryl amine resins for the production of C-terminal carboxamides. For the production of C-terminal acids, the corre-

sponding pyridine-2-aldoxime methiodide resin is used. Asparagine, glutamine, and arginine are coupled using pre-formed hydroxy benzotriazole esters. The following side chain protection may be used:

Arg,	Tosyl
Asp,	cyclohexyl
Glu,	cyclohexyl
Ser,	Benzyl
Thr,	Benzyl
Tyr,	4-bromo carbobenzoxy

Removal of the t-butoxycarbonyl moiety (deprotection) may be accomplished with trifluoroacetic acid (TFA) in methylene chloride. Following completion of the synthesis the peptides may be deprotected and cleaved from the resin with anhydrous hydrogen fluoride containing 10% meta-cresol. Cleavage of the side chain protecting group(s) and of the peptide from the resin is carried out at zero degrees centigrade or below, preferably -20°C for thirty minutes followed by thirty minutes at 0°C.

After removal of the hydrogen fluoride, the peptide/resin is washed with ether, and the peptide extracted with glacial acetic acid and then lyophilized. Purification is accomplished by size-exclusion chromatography on a Sephadex G-10 (Pharmacia) column in 10% acetic acid.

The proteins of the present invention may also be produced by recombinant methods. Recombinant methods are preferred if a high yield is desired. A general method for the construction of any desired DNA sequence is provided in J. Brown, *et al.*, Methods in Enzymology, 68:109 (1979). See also, J. Sambrook, *et al.*, supra.

The basic steps in the recombinant production of desired proteins are:

- a) construction of a synthetic or semi-synthetic DNA encoding the protein of interest;
- b) integrating said DNA into an expression vector in a manner suitable for the expression of the protein of interest, either alone or as a fusion protein;
- c) transforming an appropriate eukaryotic or prokaryotic host cell with said expression vector,
- d) culturing said transformed or transfected host cell in a manner to express the protein of interest; and
- e) recovering and purifying the recombinantly produced protein of interest.

In general, prokaryotes are used for cloning of DNA sequences in constructing the vectors of this invention. Prokaryotes may also be employed in the production of the protein of interest. For example, the *Escherichia coli* K12 strain 294 (ATCC No. 31446) is particularly useful for the prokaryotic expression of foreign proteins. Other strains of *E. coli* which may be used (and their relevant genotypes) include the following.

Strain	Genotype
DH5 α	F ⁻ (ϕ 80dlacZ Δ M15), Δ (lacZYA-argF)U169 supE44, λ^- , hsdR17 (r_K^- , m_K^+), recA1, endA1, gyrA96, thi-1, relA1
HB101	supE44, hsdS20(r_B^- m_B^-), recA13, ara14, proA2 lacY1, galK2, rpsL20, xyl-5, mtl-1, mcrB, mrr
JM109	recA1, e14 ⁻ (mcrA), supE44, endA1, hsdR17(r_K^- , m_K^+), gyrA96, relA1, thi-1, Δ (lac-proAB), F'[traD36, proAB+ lacI ^q , lacZ Δ M15]
RR1	supE44, hsdS20(r_B^- m_B^-), ara-14 proA2, lacY1, galK2, rpsL20, xyl-5, mtl-5
χ 1776	F ⁻ , ton, A53, dapD8, minA1, supE42 (glnV42), Δ (gal-uvrB)40, minB2, rfb-2, gyrA25, thyA142, oms-2, metC65, oms-1, Δ (bioH-asd)29, cycB2, cycA1, hsdR2, λ^-
294	endA, thi ⁻ , hsr ⁻ , hsm _K ⁺ (U.S. Patent 4, 366,246)

These strains are all commercially available from suppliers such as: Bethesda Research Laboratories, Gaithersburg, Maryland 20877 and Stratagene Cloning Systems, La Jolla, California 92037; or are readily available to the public from sources such as the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, 10852-1776.

Except where otherwise noted, these bacterial strains can be used interchangeably. The genotypes listed are illustrative of many of the desired characteristics for choosing a bacterial host and are not meant to limit the invention

in any way. The genotype designations are in accordance with standard nomenclature. See, for example, J. Sambrook, et al., supra. A preferred strain of E. coli employed in the cloning and expression of the genes of this invention is RV308, which is available from the ATCC under accession number ATCC 31608, and is described in United States Patent 4,551,433, issued November 5, 1985.

In addition to the strains of E. coli discussed supra, bacilli such as Bacillus subtilis, other enterobacteriaceae such as Salmonella typhimurium or Serratia marcescans, and various Pseudomonas species may be used. In addition to these gram-negative bacteria, other bacteria, especially Streptomyces, spp., may be employed in the prokaryotic cloning and expression of the proteins of this invention.

Promoters suitable for use with prokaryotic hosts include the β -lactamase [vector pGX2907 (ATCC 39344) contains the replicon and β -lactamase gene] and lactose promoter systems [Chang et al., Nature (London), 275:615 (1978); and Goeddel et al., Nature (London), 281:544 (1979)], alkaline phosphatase, the tryptophan (trp) promoter system [vector pATH1 ATCC 37695] is designed to facilitate expression of an open reading frame as a trpE fusion protein under control of the trp promoter] and hybrid promoters such as the tac promoter (isolatable from plasmid pDR540 ATCC-37282). However, other functional bacterial promoters, whose nucleotide sequences are generally known, enable one of skill in the art to ligate them to DNA encoding the proteins of the instant invention using linkers or adapters to supply any required restriction sites. Promoters for use in bacterial systems also will contain a Shine-Dalgarno sequence operably linked to the DNA encoding the desired polypeptides. These examples are illustrative rather than limiting.

The proteins of this invention may be synthesized either by direct expression or as a fusion protein comprising the protein of interest as a translational fusion with another protein or peptide which may be removable by enzymatic or chemical cleavage. It is often observed in the production of certain peptides in recombinant systems that expression as a fusion protein prolongs the lifespan, increases the yield of the desired peptide, or provides a convenient means of purifying the protein of interest. A variety of peptidases (e.g. trypsin) which cleave a polypeptide at specific sites or digest the peptides from the amino or carboxy termini (e.g. diaminopeptidase) of the peptide chain are known. Furthermore, particular chemicals (e.g. cyanogen bromide) will cleave a polypeptide chain at specific sites. The skilled artisan will appreciate the modifications necessary to the amino acid sequence (and synthetic or semi-synthetic coding sequence if recombinant means are employed) to incorporate site-specific internal cleavage sites. See e.g., P. Carter, "Site Specific Proteolysis of Fusion Proteins", Chapter 13 in Protein Purification: From Molecular Mechanisms to Large Scale Processes, American Chemical Society, Washington, D.C. (1990).

In addition to cloning and expressing the genes of interest in the prokaryotic systems discussed above, the proteins of the present invention may also be produced in eukaryotic systems. The present invention is not limited to use in a particular eukaryotic host cell. A variety of eukaryotic host cells are available from depositories such as the American Type Culture Collection (ATCC) and are suitable for use with the vectors of the present invention. The choice of a particular host cell depends to some extent on the particular expression vector used to drive expression of the human glutamate receptor-encoding nucleic acids of the present invention. Exemplary host cells suitable for use in the present invention are listed in Table I

Table I

Host Cell	Origin	Source
HepG-2	Human Liver Hepatoblastoma	ATCC HB 8065
CV-1	African Green Monkey Kidney	ATCC CCL 70
LLC-MK ₂	Rhesus Monkey Kidney	ATCC CCL 7.1
3T3	Mouse Embryo Fibroblasts	ATCC CCL 92
CHO-K1	Chinese Hamster Ovary	ATCC CCL 61
HeLa	Human Cervix Epitheloid	ATCC CCL 2
RPMI8226	Human Myeloma	ATCC CCL 155
H4IIEC3	Rat Hepatoma	ATCC CCL 1600
C1271	Mouse Fibroblast	ATCC CCL 1616
HS-Sultan	Human Plasma Cell Plasmacytoma	ATCC CCL 1484
BHK-21	Baby Hamster Kidney	ATCC CCL 10

An especially preferred cell line employed in this invention is the widely available cell line AV12-664 (hereinafter "AV12"). This cell line is available from the American Type Culture Collection under the accession number ATCC CRL 9595. The AV12 cell line was constructed by injecting a Syrian hamster in the scruff of the neck with human adenovirus 12 and isolating cells from the resulting tumor.

A wide variety of vectors, some of which are discussed below, exists for the transformation of such mammalian

host cells, but the specific vectors described herein are in no way intended to limit the scope of the present invention.

The pSV2-type vectors comprise segments of the simian virus 40 (SV40) genome that constitute a defined eukaryotic transcription unit-promoter, intervening sequence, and polyadenylation site. In the absence of the SV40 T antigen, the plasmid pSV2-type vectors transform mammalian and other eukaryotic host cells by integrating into the host cell chromosomal DNA. A large number of plasmid pSV2-type vectors have been constructed, such as plasmid pSV2-gpt, pSV2-neo, pSV2-dhfr, pSV2-hyg, and pSV2- β -globin, in which the SV40 promoter drives transcription of an inserted gene. These vectors are suitable for use with the coding sequences of the present invention and are widely available from sources such as the ATCC or the Northern Regional Research Laboratory (NRRL), 1815 N. University Street, Peoria, Illinois, 61604.

The plasmid pSV2-dhfr (ATCC 37146) comprises a murine dihydrofolate reductase (dhfr) gene under the control of the SV40 early promoter. Under the appropriate conditions, the dhfr gene is known to be amplified, or copied, in the host chromosome. This amplification can result in the amplification of closely-associated DNA sequences and can, therefore, be used to increase production of a protein of interest. See, e.g., J. Schimke, *Cell*, 35:705-713 (1984).

Plasmids constructed for expression of the proteins of the present invention in mammalian and other eukaryotic host cells can utilize a wide variety of promoters. The present invention is in no way limited to the use of the particular promoters exemplified herein. Promoters such as the SV40 late promoter, promoters from eukaryotic genes, such as, for example, the estrogen-inducible chicken ovalbumin gene, the interferon genes, the glucocorticoid-inducible tyrosine aminotransferase gene, and the thymidine kinase gene, and the major early and late adenovirus genes can be readily isolated and modified to express the genes of the present invention. Eukaryotic promoters can also be used in tandem to drive expression of a coding sequence of this invention. Furthermore, a large number of retroviruses are known that infect a wide range of eukaryotic host cells. The long terminal repeats in the retroviral DNA frequently encode functional promoters and, therefore, may be used to drive expression of the nucleic acids of the present invention.

Plasmid pRSVcat (ATCC 37152) comprises portions of a long terminal repeat of the Rous Sarcoma virus, a virus known to infect chickens and other host cells. This long terminal repeat contains a promoter which is suitable for use in the vectors of this invention. H. Gorman, *et al.*, *Proceedings of the National Academy of Sciences (USA)*, 79:6777 (1982). The plasmid pMSVi (NRRL B-15929) comprises the long terminal repeats of the Murine Sarcoma virus, a virus known to infect mouse and other host cells. The mouse metallothionein promoter has also been well characterized for use in eukaryotic host cells and is suitable for use in the expression of the nucleic acids of the present invention. The mouse metallothionein promoter is present in the plasmid pDBPV,MMTneo (ATCC 37224) which can serve as the starting material of other plasmids of the present invention.

An especially preferred expression vector system employs one of a series of vectors containing the BK enhancer, an enhancer derived from the BK virus, a human papovavirus. The most preferred such vector systems are those which employ not only the BK enhancer but also the adenovirus-2-early region 1A (E1A) gene product. The E1A gene product (actually, the E1A gene produces two products, which are collectively referred to herein as "the E1A gene product") is an immediate-early gene product of adenovirus, a large DNA virus.

A most preferred expression vector employed in the present invention is the phd series of vectors which comprise a BK enhancer in tandem with the adenovirus late promoter to drive expression of useful products in eukaryotic host cells. The construction and method of using the phd plasmid, as well as related plasmids, are described in U.S. Patents 5,242,688, issued September 7, 1993, and 4,992,373, issued February 12, 1991, as well as co-pending United States patent application 07/368,700, all of which are herein incorporated by reference. *Escherichia coli* K12 GM48 cells harboring the plasmid phd are available as part of the permanent stock collection of the Northern Regional Research Laboratory under accession number NRRL B-18525. The plasmid may be isolated from this culture using standard techniques.

The plasmid phd contains a unique *Bcl*I site which may be utilized for the insertion of the gene encoding the protein of interest. The skilled artisan understands that linkers or adapters may be employed in cloning the gene of interest into this *Bcl*I site. A depiction of the plasmid phd is provided as Figure 2 of this document. The phd series of plasmids functions most efficiently when introduced into a host cell which produces the E1A gene product, cell lines such as AV12-664, 293 cells, and others, described *supra*.

Transformation of the mammalian cells can be performed by any of the known processes including, but not limited to, the protoplast fusion method, the calcium phosphate co-precipitation method, electroporation and the like. See, e.g., J. Sambrook, *et al.*, *supra*, at 3:16.30-3:16.66.

Other routes of production are well known to skilled artisans. In addition to the plasmid discussed above, it is well known in the art that some viruses are also appropriate vectors. For example, the adenovirus, the adeno-associated virus, the vaccinia virus, the herpes virus, the baculovirus, and the rous sarcoma virus are useful. Such a method is described in U.S. Patent 4,775,624, herein incorporated by reference. Several alternate methods of expression are described in J. Sambrook, *et al.*, *supra*, at 16.3-17.44.

In addition to prokaryotes and mammalian host cells, eukaryotic microbes such as yeast cultures may also be

used. The imperfect fungus Saccharomyces cerevisiae, or common baker's yeast, is the most commonly used eukaryotic microorganism, although a number of other strains are commonly available. For expression in Saccharomyces sp., the plasmid YRp7 (ATCC-40053), for example, is commonly used. See, e.g., L. Stinchcomb, et al., Nature, 282: 39 (1979); J. Kingsman et al., Gene, 7:141 (1979); S. Tschemper et al., Gene, 10:157 (1980). This plasmid already contains the trp gene which provides a selectable marker for a mutant strain of yeast lacking the ability to grow in tryptophan.

Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [found on plasmid pAP12BD (ATCC 53231) and described in U.S. Patent No. 4,935,350, issued June 19, 1990, herein incorporated by reference] or other glycolytic enzymes such as enolase [found on plasmid pAC1 (ATCC 39532)], glyceraldehyde-3-phosphate dehydrogenase [derived from plasmid pHcGAPC1 (ATCC 57090, 57091)], hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase, as well as the alcohol dehydrogenase and pyruvate decarboxylase genes of Zymomonas mobilis (United States Patent No. 5,000,000 issued March 19, 1991, herein incorporated by reference).

Other yeast promoters, which are inducible promoters, having the additional advantage of their transcription being controllable by varying growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein [contained on plasmid vector pCL28XhoLHBPV (ATCC 39475) and described in United States Patent No. 4,840,896, herein incorporated by reference], glyceraldehyde 3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose [e.g. GAL1 found on plasmid pRY121 (ATCC 37658)] utilization. Suitable vectors and promoters for use in yeast expression are further described in R. Hitzeman et al., European Patent Publication No. 73,657A. Yeast enhancers such as the UAS Gal from Saccharomyces cerevisiae (found in conjunction with the CYC1 promoter on plasmid YEpsc--hllbeta ATCC 67024), also are advantageously used with yeast promoters.

Practitioners of this invention realize that, in addition to the above-mentioned expression systems, the cloned cDNA may also be employed in the production of transgenic animals in which a test mammal, usually a mouse, in which expression or overexpression of the proteins of the present invention can be assessed. The nucleic acids of the present invention may also be employed in the construction of "knockout" animals in which the expression of the native cognate of the gene is suppressed.

Skilled artisans also recognize that some alterations of SEQ ID NO:2 will fail to change the function of the amino acid compound. For instance, some hydrophobic amino acids may be exchanged for other hydrophobic amino acids. Those altered amino acid compounds which confer substantially the same function in substantially the same manner as the exemplified amino acid compound are also encompassed within the present invention. Typical such conservative substitutions attempt to preserve the: (a) secondary or tertiary structure of the polypeptide backbone; (b) the charge or hydrophobicity of the residue; or (c) the bulk of the side chain. Some examples of such conservative substitutions of amino acids, resulting in the production of proteins which are functional equivalents of the protein of SEQ ID NO:2 are shown in Table II, infra.

Table II

Original Residue	Exemplary Substitutions
Ala	Ser, Gly
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro, Ala
His	Asn, Gln
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile
Phe	Met, Leu, Tyr
Ser	Thr

Continuation of the Table on the next page

Table II (continued)

Original Residue	Exemplary Substitutions
Thr	Ser
Trp	Tyr
Tyr	Trp, Phe
Val	Ile, Leu

These substitutions may be introduced into the protein in a variety of ways, such as during the chemical synthesis or by chemical modification of an amino acid side chain after the protein has been prepared.

Alterations of the protein having a sequence which corresponds to the sequence of SEQ ID NO:2 may also be induced by alterations of the nucleic acid compounds which encodes these proteins. These mutations of the nucleic acid compound may be generated by either random mutagenesis techniques, such as those techniques employing chemical mutagens, or by site-specific mutagenesis employing oligonucleotides. Those nucleic acid compounds which confer substantially the same function in substantially the same manner as the exemplified nucleic acid compounds are also encompassed within the present invention.

Other embodiments of the present invention are nucleic acid compounds which comprise isolated nucleic acid sequences which encode SEQ ID NO:2. As skilled artisans will recognize, the amino acid compounds of the invention can be encoded by a multitude of different nucleic acid sequences because most of the amino acids are encoded by more than one nucleic acid triplet due to the degeneracy of the amino acid code. Because these alternative nucleic acid sequences would encode the same amino acid sequences, the present invention further comprises these alternate nucleic acid sequences.

The gene encoding the human glutamate mGluR2 receptor molecule may be produced using synthetic methodology. This synthesis of nucleic acids is well known in the art. See, e.g., E.L. Brown, R. Belagaje, M.J. Ryan, and H. G. Khorana, Methods in Enzymology, 68:109-151 (1979). The DNA segments corresponding to the receptor gene are generated using conventional DNA synthesizing apparatus such as the Applied Biosystems Model 380A or 380B DNA synthesizers (commercially available from Applied Biosystems, Inc., 850 Lincoln Center Drive, Foster City, CA 94404) which employ phosphoramidite chemistry. In the alternative, the more traditional phosphotriester chemistry may be employed to synthesize the nucleic acids of this invention. [See, e.g., M.J. Gait, ed., Oligonucleotide Synthesis. A Practical Approach, (1984).]

The synthetic human glutamate mGluR2 receptor gene may be designed to possess restriction endonuclease cleavage sites at either end of the transcript to facilitate isolation from and integration into expression and amplification plasmids. The choice of restriction sites are chosen so as to properly orient the coding sequence of the receptor with control sequences to achieve proper in-frame reading and expression of the mGluR2 receptor molecule. A variety of other such cleavage sites may be incorporated depending on the particular plasmid constructs employed and may be generated by techniques well known in the art.

In an alternative methodology, the desired DNA sequences can be generated using the polymerase chain reaction as described in U.S. Patent No. 4,889,818, which is herein incorporated by reference.

In addition to the deoxyribonucleic acid of SEQ ID NO:1, this invention also provides ribonucleic acids (RNA) which comprise the RNA sequence

EP 0 711 832 A2

	AUGGGAUCGC UGCUUGCGCU CCUGGCACUG CUGCCGCUGU GGGGUGCUGU	50
	GGCUGAGGGC CCAGCCAAGA AGGUGCUGAC CCUGGAGGGA GACUUGGUGC	100
5	UGGGUGGGCU GTUCCAGUG CACCAGAAGG GCGGCCCAGC AGAGGACUGU	150
	GGUCCUGUCA AUGAGCACCG UGGCAUCCAG CGCCUGGAGG CCAUGCUUUU	200
10	UGCACUGGAC CGCAUCAACC GUGACCCGCA CCUGCUGCCU GGCUGGCGCC	250
	UGGGUGCACA CAUCCUCGAC AGUUGCUGCA AGGACACACA UGCGCUGGAG	300
	CAGGCACUGG ACUUUGUGCG UGCCUCACUC AGCCGUGGUG CUGAUGGCUC	350
15	ACGCCACAUC UGCCCCGACG GCUCUUAUGC GACCCAUGGU GAUGCUGCCA	400
	CUGCCAUCAC UGGUGUUAUU GCGGUGUCCU ACAGUGAUGU CUCCAUCCAG	450
	GUGGCCAACC UCUGAGGCU AUUUCAGAU CACAGAUUA GCUACGCCUC	500
20	UACCAGUGCC AAGCUGAGUG ACAAGUCCCG CUAUGACUAC UUUGCCCCGCA	550
	CAGUGCCUCC UGACUUCUUC CAAGCCAAGG CCAUGGCUGA GAUUCUCCGC	600
25	UUUUCAACU GGACCUAUGU GUCCACUGUG GCGUCUGAGG GCGACUAUGG	650
	CGAGACAGGC AUUGAGGCCU UUGAGCUAGA GGCUCGUGCC CGCAACAUCU	700
	GUGUGGCCAC CUCGGAGAAA GUGGGCCGUG CCAUGAGCCG CGCGGCCUUU	750
30	GAGGGUGUGG UGCGAGCCCU GCUGCAGAAG CCCAGUGCCC GCGUGGCUGU	800
	CCUGUUCACC CGUUCUGAGG AUGCCCGGGA GCUGCUUGCU GCCAGCCAGC	850
	GCCUCAAUGC CAGCUUCACC UGGGUGGCCA GUGAUGGUUG GGGGGCCCUG	900
35	GAGAGUGUGG UGGCAGGCAG UGAGGGGGCU GCUGAGGGUG CUAUCACCAU	950
	CGAGCUGGCC UCCUACCCCA UCAGUGACUU UGCCUCCUAC UUCAGAGCC	1000
40	UGGACCCUUG GAACAACAGC CGGAACCCCU GGUUCCGUGA AUUCUGGGAG	1050
45		
50		
55		

EP 0 711 832 A2

	CAGAGGUUCC GCUGCAGCUU CCGGCAGCGA GACUGCGCAG CCCACUCUCU	1100
5	CCGGGCUGUG CCCUUUGAGC AGGAGUCCAA GAUCAUGUUU GUGGUCAAUG	1150
	CAGUGUACGC CAUGGCCCAU GCGCUCCACA ACAUGCACCG UGCCCUCUGC	1200
	CCCAACACCA CCCGGCUCUG UGACGCGAUG CGGCCAGUUA ACGGGCGCCG	1250
10	CCUCUACAAG GACUUUGUGC UCAACGUCAA GUUUGAUGCC CCCUUUCGCC	1300
	CAGCUGACAC CCACAAUGAG GUCCGCUUUG ACCGCUUUGG UGAUGGUAUU	1350
	GGCCGCUACA ACAUCUUCAC CUAUCUGCGU GCAGGCAGUG GGCGCUAUCG	1400
15	CUACCAGAAG GUGGGCUACU GGGCAGAAGG CUUGACUCUG GACACCAGCC	1450
	UCAUCCCAUG GGCCUCACCC UCAGCCGGCC CCCUGCCCGC CUCUCGCUGC	1500
	AGUGAGCCCU GCCUCCAGAA UGAGGUGAAG AGUGUGCAGC CGGGCGAAGU	1550
20	CUGCUGCUGG CUCUGCAUUC CGUGCCAGCC CUAUGAGUAC CGAUUGGACG	1600
	AAUUCACUUG CGCUGAUUGU GGCCUGGGCU ACUGGCCCAA UGCCAGCCUG	1650
25	ACUGGCUGCU UCGAACUGCC CCAGGAGUAC AUCCGCUGGG GCGAUGCCUG	1700
	GGCUGUGGGA CCUGUCACCA UCGCCUGCCU CGGUGCCCUG GCCACCCUCU	1750
	UUGUGCUGGG UGUCUUUGUG CGGCACAAUG CCACACCAGU GGUCAAGGCC	1800
30	UCAGGUCGGG AGCUCUGCUA CAUCCUGCUG GGUGGUGUCU UCCUCUGCUA	1850
	CUGCAUGACC UUCAUCUUA UUGCCAAGCC AUCCACGGGA GUGUGUGCCU	1900
	UACGGCGUCU UGGGGUGGGC ACUGCCUUCU CUGUCUGCUA CUCAGCCCUG	1950
35	CUCACCAAGA CCAACCGCAU UGCACGCAUC UUCGGUGGGG CCCGGGAGGG	2000
	UGCCCAGCGG CCACGCUUCA UCAGUCCUGC CUCACAGGUG GCCAUCUGCC	2050
	UGGCACUUAU CUCGGGCCAG CUGCUCAUCG UGGUCGCCUG GCUGGUGGUG	2100
40	GAGGCACCGG GCACAGGCAA GGAGACAGCC CCCGAACGGC GGGAGGUGGU	2150
	GACACUGCGC UGCAACCACC GCGAUGCAAG UAUGUUGGGC UCGCUGGCCU	2200
	ACAAUGUGCU CCUCAUCGCG CUCUGCACGC UUAUGCCUU CAAGACUCGC	2250
45	AAGUGCCCCG AAAACUUCAA CGAGGCCAAG UUCAUUGGCU UCACCAUGUA	2300
	CACCACCUGC AUCAUCUGGC UGGCAUUCUU GCCCAUCUUC UAUGUCACCU	2350
50	CCAGUGACUA CCGGUACAG ACCACCACCA UGUGCGUGUC AGUCAGCCUC	2400
	AGCGGCUCGG UGGUGCUUGG CUGCCUCUUU GCGCCAAGC UGCACAUCAU	2450
	CCUCUCCAG CCGCAGAAGA ACGUGGUUAG CCACCGGGCA CCCACCAGCC	2500
55		

GCUUUGGCAG UGCUGCUGCC AGGGCCAGCU CCAGCCUUGG CCAAGGGUCU 2550
 GGCUCCCAGU UUGUCCCCAC UGUUUGCAAU GGCCGUGAGG UGGUGGACUC 2600
 5 GACAACGUCA UCGCUUUGA 2619

hereinafter referred to as SEQ ID NO:3, or the complementary ribonucleic acid, or a fragment of either SEQ ID NO:3
 or the complement thereof. The ribonucleic acids of the present invention may be prepared using the polynucleotide
 10 synthetic methods discussed supra or they may be prepared enzymatically using RNA polymerases to transcribe a
 DNA template. complement thereof.

The most preferred systems for preparing the ribonucleic acids of the present invention employ the RNA polymer-
 ase from the bacteriophage T7 or the bacteriophage SP6. Both of these RNA polymerases are highly specific and
 require the insertion of bacteriophage-specific sequences at the 5' end of the message to be read. See, J. Sambrook,
 15 et al., supra, at 18.82-18.84.

This invention also provides nucleic acids, RNA or DNA, which are complementary to SEQ ID NO:1 or SEQ ID NO:3.

The present invention also provides probes and primers useful for molecular biology techniques. A compound
 which encodes for SEQ ID NO:1, SEQ ID NO:3 or a complementary sequence of SEQ ID NO:1 or SEQ ID NO:3, or a
 fragment thereof, and which is at least 18 base pairs in length, and which will selectively hybridize to human genomic
 20 DNA or messenger RNA encoding a human glutamate receptor, is provided. Preferably, the 18 or more base pair
 compound is DNA.

The term "selectively hybridize" as used herein may refer to either of two situations. In the first such embodiment
 of this invention, the nucleic acid compounds described supra hybridize to a human glutamate receptor under more
 stringent hybridization conditions than these same nucleic acid compounds would hybridize to an analogous glutamate
 25 receptor of another species, e.g. murine or primate. In the second such embodiment of this invention, these probes
 hybridize to the mGluR2 receptor under more stringent hybridization conditions than other related compounds, including
 nucleic acid sequences encoding other glutamate receptors.

These probes and primers can be prepared enzymatically as described supra. In a most preferred embodiment
 these probes and primers are synthesized using chemical means as described supra. Probes and primers of defined
 30 structure may also be purchased commercially.

This invention also encompasses recombinant DNA cloning vectors and expression vectors comprising the nucleic
 acids of the present invention. Many of the vectors encompassed within this invention are described above. The pre-
 ferred nucleic acid vectors are those which are DNA. The most preferred recombinant DNA vector comprises the
 isolated DNA sequence SEQ ID NO:1. Plasmid pRS133, which has been deposited with the NRRL and is available
 35 under accession number NRRL B-21174, is an especially preferred DNA vector of the present invention.

The sequence of SEQ ID NO:1 was prepared from a human fetal brain cDNA library (commercially available from
 Stratagene, Inc.). An aliquot of this library was used as a template with short synthetic oligonucleotide primers, designed
 by evaluation of the DNA sequences of the rat metabotropic receptors described in Tanabe, et al., Neuron, 8:169-172
 (1992). Use of these oligonucleotides with the polymerase chain reaction generated a 1.5 kb DNA fragment, which
 40 was gel purified, radioactively labeled by PCR and used as a probe to screen the cDNA library for individual human
 mGluR2 clones. Using standard plaque hybridization techniques (moderate stringency, 1 M Na⁺, 60°C) a number of
 positive clones were isolated. By further dilution and hybridization, a phage clone was purified which contained the
 complete human mGluR2 sequence on a 2.8 kb DNA insert. A plasmid containing the insert was excised from the
 phage using helper phage and protocols supplied by the manufacturer. The cDNA fragment was removed from this
 45 plasmid on a 2.8 kb XbaI and BglII fragment.

The 2.8 kb fragment obtained was first subcloned into plasmid pSL301 (generating pRS133, as depicted in Figure
 1) in order to allow its subsequent removal on a BamHI fragment. This fragment was then inserted into the BclI site of
 the mammalian expression vector phd, yielding the plasmid pRS138 (Figure 2).

The skilled artisan understands that the type of cloning vector or expression vector employed depends upon the
 50 availability of appropriate restriction sites, the type of host cell in which the vector is to be transfected or transformed,
 the purpose of the transfection or transformation (e.g., transient expression in an oocyte system, stable transformation
 as an extrachromosomal element, or integration into the host chromosome), the presence or absence of readily as-
 sayable markers (e.g., antibiotic resistance markers, metabolic markers, or the like), and the number of copies of the
 gene to be present in the cell.

The type of vector employed to carry the nucleic acids of the present invention may be RNA viruses, DNA viruses,
 55 lytic bacteriophages, lysogenic bacteriophages, stable bacteriophages, plasmids, viroids, and the like. The most pre-
 ferred vectors of the present invention are those derived from plasmids.

When preparing an expression vector the skilled artisan understands that there are many variables to be consid-

ered. One such example is the use of a constitutive promoter, i.e. a promoter which is functional at all times, instead of a regulatable promoter which may be activated or inactivated by the artisan using heat, addition or removal of a nutrient, addition of an antibiotic, and the like. The practitioner also understands that the amount of nucleic acid or protein to be produced dictates, in part, the selection of the expression system. For experiments examining the amount of the protein expressed on the cell membrane or for experiments examining the biological function of an expressed membrane protein, for example, it may be unwise to employ an expression system which produces too much of the protein. The addition or subtraction of certain sequences, such as a signal sequence preceding the coding sequence, may be employed by the practitioner to influence localization of the resulting polypeptide. Such sequences added to or removed from the nucleic acid compounds of the present invention are encompassed within this invention.

Plasmid pRS133 may be isolated from the deposited *E. coli* containing these plasmids using standard procedures such as cesium chloride DNA isolation. Cleaving of pRS133 with the restriction enzyme *Bam*HI yields a four kilobasepair fragment comprising SEQ ID NO:2. The relative locations of these restriction sites and the direction of translation of the proteins of the instant invention are depicted in Figures 1 and 2.

The plasmid pRS133 is readily modified to construct expression vectors that produce mGluR2 receptors in a variety of organisms, including, for example, *E. coli*, Sf9 (as host for baculovirus), *Spodoptera* and *Saccharomyces*. The current literature contains techniques for constructing AV12 expression vectors and for transforming AV12 host cells. United States Patent No. 4,992,373, herein incorporated by reference, is one of many references describing these techniques.

One of the most widely employed techniques for altering a nucleic acid sequence is by way of oligonucleotide-directed site-specific mutagenesis. B. Comack, "Current Protocols in Molecular Biology", 8.01-8.5.9, (F. Ausubel, et al., eds. 1991). In this technique an oligonucleotide, whose sequence contains the mutation of interest, is synthesized as described *supra*. This oligonucleotide is then hybridized to a template containing the wild-type sequence. In a most preferred embodiment of this technique, the template is a single-stranded template. Particularly preferred are plasmids which contain regions such as the fl intergenic region. This region allows the generation of single-stranded templates when a helper phage is added to the culture harboring the "phagemid".

After the annealing of the oligonucleotide to the template, a DNA-dependent DNA polymerase is then used to synthesize the second strand from the oligonucleotide, complementary to the template DNA. The resulting product is a heteroduplex molecule containing a mismatch due to the mutation in the oligonucleotide. After DNA replication by the host cell a mixture of two types of plasmid are present, the wild-type and the newly constructed mutant. This technique permits the introduction of convenient restriction sites such that the coding sequence may be placed immediately adjacent to whichever transcriptional or translational regulatory elements are employed by the practitioner.

The construction protocols utilized for *E. coli* can be followed to construct analogous vectors for other organisms, merely by substituting, if necessary, the appropriate regulatory elements using techniques well known to skilled artisans.

Host cells which harbor the nucleic acids provided by the present invention are also provided. A preferred host cell is an *Xenopus* sp. oocyte which has been injected with RNA or DNA compounds of the present invention. Most preferred oocytes of the present invention are those which harbor a sense mRNA of the present invention. Other preferred host cells include AV12 and *E. coli* cells which have been transfected and/or transformed with a vector which comprises a nucleic acid of the present invention.

The present invention also provides a method for constructing a recombinant host cell capable of expressing SEQ ID NO:2, said method comprising transforming a host cell with a recombinant DNA vector that comprises an isolated DNA sequence which encodes SEQ ID NO:2. The preferred host cell is AV12. The preferred vector for expression is one which comprises SEQ ID NO:1. Another preferred host cell for this method is *E. coli*. An especially preferred expression vector in *E. coli* is one which comprises SEQ ID NO:1. Transformed host cells may be cultured under conditions well known to skilled artisans such that SEQ ID NO:2 is expressed, thereby producing mGluR2 in the recombinant host cell.

The ability of glutamate to bind to the mGluR2 receptor is essential in the development of a multitude of indications. In developing agents which act as antagonists or agonists of the mGluR2 receptor, it would be desirable, therefore, to determine those agents which bind the mGluR2 receptor. Generally, such an assay includes a method for determining whether a substance is a functional ligand of the mGluR2 receptor, said method comprising contacting a functional compound of the mGluR2 receptor with said substance, monitoring binding activity by physically detectable means, and identifying those substances which effect a chosen response. Preferably, the physically detectable means is competition with labeled glutamate or binding of ligand in an oocyte transient expression system.

The instant invention provides such a screening system useful for discovering agents which compete with glutamate for binding to the mGluR2 receptor, said screening system comprising the steps of:

- a) isolating a human mGluR2 receptor;
- b) exposing said human mGluR2 receptor to a potential inhibitor or surrogate of the glutamate/mGluR2 receptor complex;

- c) introducing glutamate;
- d) removing non-specifically bound molecules; and
- 5 e) quantifying the concentration of bound potential inhibitor and/or glutamate.

This allows one to rapidly screen for inhibitors or surrogates of the formation of the glutamate/mGluR2 receptor complex. Utilization of the screening system described above provides a sensitive and rapid means to determine compounds which interfere with the formation of the glutamate/mGluR2 receptor complex. This screening system may also
10 be adapted to automated procedures such as a PANDEX® (Baxter-Dade Diagnostics) system allowing for efficient high-volume screening of potential therapeutic agents.

In such a screening protocol a mGluR2 receptor is prepared as elsewhere described herein, preferably using recombinant DNA technology. A sample of a test compound is then introduced to the reaction vessel containing the mGluR2 receptor followed by the addition of glutamate. In the alternative the glutamate may be added simultaneously
15 with the test compound. Unbound molecules are washed free and the eluent inspected for the presence of glutamate or the test compound.

For example, in a preferred method of the invention, radioactively or chemically labeled glutamate may be used. The eluent is then scored for the chemical label or radioactivity. The absence or diminution of the chemical label or radioactivity indicates the formation of the glutamate/mGluR2 receptor complex. This indicates that the test compound
20 has not effectively competed with glutamate in the formation of the glutamate/mGluR2 receptor complex. The presence of the chemical label or radioactivity indicates that the test compound has competed with glutamate in the formation of the glutamate/mGluR2 receptor complex. Similarly, a radioactively or chemically labeled test compound may be used in which case the same steps as outlined above would be used except that the interpretation of results would be the converse of using radioactively or chemically labelled glutamate.

As would be understood by the skilled artisan these assays may also be performed such that the practitioner measures the radioactivity or fluorescence remaining with the protein, not in the eluent. A preferred such assay employs radiolabeled glutamate. After the competition reaction has been performed the reaction mixture is then passed through a filter, the filter retaining the receptor and whatever is complexed with the receptor. The radioactivity on each filter is then measured in a scintillation counter. In such an assay higher amounts of radiolabel present indicate lower affinity
25 for the receptor by the test compound.

The mGluR2 receptor may be free in solution or bound to a solid support. Whether the mGluR2 receptor is bound to a support or is free in solution, it is generally important that the conformation of the protein be conserved. In a preferred practice of the invention, therefore, the mGluR2 receptor is suspended in a hydrophobic environment employing natural or synthetic detergents, membrane suspensions, and the like. Preferred detergent complexes include
35 the zwitterionic detergent 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate ("CHAPS") as well as sodium deoxycholate.

Skilled artisans will recognize that desirable dissociation constant (K_i) values are dependent on the selectivity of the compound tested. For example, a compound with a K_i which is less than 10 nM is generally considered an excellent candidate for drug therapy. However, a compound which has a lower affinity, but is selective for the particular receptor,
40 may be an even better candidate. The present invention, however, provides radiolabeled competition assays, whether results therefrom indicate high affinity or low affinity to mGluR2 receptor, because skilled artisans will recognize that any information regarding binding or selectivity of a particular compound is beneficial in the pharmaceutical development of drugs.

In one such competition assay, a battery of known glutamate receptor antagonists, agonists, and partial agonists
45 are evaluated for their relative abilities to inhibit the binding of [3 H]glutamate to the human mGluR2 receptor of the present invention.

In this assay suspension cells stably expressing the cloned human mGluR2 receptor are harvested by centrifugation at 2200 x g for 15 minutes at 4°C. Membranes for the binding assays are prepared by vortexing the cell pellet in 50 mM Tris.HCl, pH 7.4 (0.5 x 10⁹ cells/30 ml). The tissue suspension is then centrifuged at 39,800 x g for 10 minutes
50 at 4°C. This procedure is repeated for a total of three washes, with a 10 minute incubation at 37°C between the second and third washes. The final pellet is homogenized in 67 mM Tris.HCl, pH 7.4, at 12.5 x 10⁶ cells/ml using a Tissumizer® (Tekmar, Cincinnati, Ohio) at setting 65 for 15 seconds.

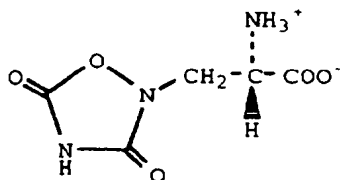
Binding assays are performed in triplicate in 0.8 ml total volume. Volumes of 200 µl of membrane suspension (0.07-0.10 mg of protein) and 200 µl of drug dilution in water are added to 400 µl of 67 mM of Tris.HCl, pH 7.4, containing
55 [3 H]glutamate (35 nM final concentration, 23.7 Ci/mole), calcium chloride (3 mM), pargyline (10 µM), and L-ascorbic acid (5.7 nM). The reaction mixtures are incubated at 37°C for 15 minutes and then rapidly filtered, using a Brandel™ cell harvester (Model MB-48R; Brandel, Gaithersburg, Maryland) over Whatman GF/B filters that had been presoaked in 0.5% polyethyleneimine and precooled with ice-cold 50 mM Tris-HCl, pH 7.4. The filters are then washed rapidly

times with ice-cold (4 x 1 ml each).

The amount of [^3H]glutamate trapped on the filters is determined by liquid scintillation counting. For the competition experiments, six concentrations of displacing drugs are used, ranging from 10^{-5} to 10^{-10} M. The IC_{50} values are determined by nonlinear regression analysis (SystatTM; Systat Inc., Evanston, Illinois) which may be converted to K_i values using the Cheng-Prusoff equation. Y. Cheng and W.H. Prusoff, *Biochemical Pharmacology*, 22:3099-3108 (1973).

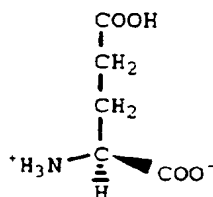
In this particular type of competition assay the following compounds are frequently used.

(a) Quisqualate -- a compound of the formula



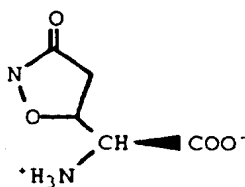
having the chemical name (S)-α-amino-3,5-dioxo-1,2,4-oxadiazolidine-2-propanoate. This compound can be prepared as described in J.E. Baldwin, *et al.*, *Chemical Communications*, 256 (1985).

(b) Glutamate -- a compound of the formula



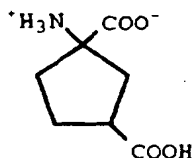
having the chemical name 1-aminopropane-1,3-dicarboxylic acid. This compound is readily available and can be purchased commercially from several sources.

(c) Ibotenate -- a compound of the formula



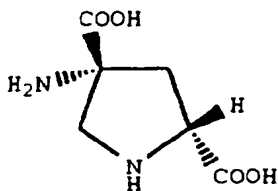
having the chemical name α-amino-3-hydroxy-5-isoxazoleacetate, which can be prepared as described in United States Patent 3,459,862, herein incorporated by reference.

(d) t-ACPD -- a compound of the formula



having the chemical name 1-aminocyclopentane-1,3-dicarboxylic acid. This compound can be purchased commercially from several sources.

(e) (2R,4R) 4-amino-pyrrolidine-2,4-dicarboxylic acid, a compound of the formula



which is described in co-pending United States Patent Application Serial Number 08/295,337, filed August 24, 1994. Many 1-substituted derivatives of this dicarboxylic acid are also effective as mGluR2 antagonists.

The previously described screening system identifies compounds which competitively bind to the mGluR2 receptor. Determination of the ability of such compounds to stimulate or inhibit the action of the mGluR2 receptor is essential to further development of such compounds for therapeutic applications. The need for a bioactivity assay system which determines the response of the mGluR2 receptor to a compound is clear. The instant invention provides such a bioactivity assay, said assay comprising the steps of:

- a) transfecting a mammalian host cell with an expression vector comprising DNA encoding a mGluR2 receptor;
- b) culturing said host cell under conditions such that the DNA encoding the mGluR2 receptor is expressed,
- c) exposing said host cell so transfected to a test compound, and
- d) measuring the change in a physiological condition known to be influenced by the binding of glutamate to the mGluR2 receptor relative to a control in which the transfected host cell is exposed to glutamate.

An oocyte transient expression system can be constructed according to the procedure described in S. Lübbert, *et al.*, Proceedings of the National Academy of Sciences (USA), 84:4332 (1987).

In an especially preferred embodiment of this invention an assay measuring the inhibition of forskolin-stimulated cAMP synthesis was performed. The inhibition of cAMP synthesis is known to be positively correlated with the addition of glutamate to cells containing certain types of metabotropic receptors.

Adenylate Cyclase Activity

Adenylate cyclase activity was determined in initial experiments in transfected mammalian cells, using standard techniques. *See, e.g.*, N. Adham, *et al.*, *supra*; R.L. Weinshank, *et al.*, Proceedings of the National Academy of Sciences (USA), 89:3630-3634 (1992), and the references cited therein.

Mammalian cells (the cell line AV12-664 was employed here) are stably transfected with the plasmid pRS138, containing human mGluR2 cDNA inserted in the plasmid vector pHD, as depicted in Figure 2. The cells are maintained in a medium consisting of Dulbecco's Modified Eagle's Medium (DMEM) containing 5% dialyzed fetal calf serum, 10 mM HEPES buffer (pH 7.3), 1 mM sodium pyruvate, 1 mM glutamine, and 200 µg/ml hygromycin.

For the assay the cells are disassociated from stock culture flasks with trypsin, and planted in 24-well plastic culture dishes (15 mm wells) at a density of 500-700,000 cells per well using the same culture medium. After twenty four hours incubation in a humidified carbon dioxide incubator, the cell monolayers are washed with buffer (Dulbecco's phosphate-buffered saline containing 0.5 mM isobutylmethylxanthine and 3 mM glucose) and then incubated in the same buffer at 37°C for 30 minutes. The monolayers are then washed four additional times with buffer.

Drugs and forskolin, or forskolin alone, dissolved in buffer, are added after the final wash. After incubating for 20 minutes at 37°C, 0.5 ml of 8 mM EDTA is added to each well. The plates are then placed in a boiling water bath for about four minutes. The supernatant fluids are then recovered from the wells and lyophilized. Cyclic adenosine monophosphate determinations are carried out on the lyophilized samples using commercially available radioimmunoassay kits, following the manufacturer's instructions. The cAMP level in wells containing drug are compared to the forskolin controls.

In another embodiment this invention provides a method for identifying, in a test sample, DNA homologous to a probe of the present invention, wherein the test nucleic acid is contacted with the probe under hybridizing conditions and identified as being homologous to the probe. Hybridization techniques are well known in the art. *See, e.g.*, J. Sambrook, *et al.*, *supra*, at Chapter 11.

The nucleic acid compounds of the present invention may also be used to hybridize to genomic DNA which has been digested with one or more restriction enzymes and run on an electrophoretic gel. The hybridization of radiolabeled

probes onto such restricted DNA, usually fixed to a membrane after electrophoresis, is well known in the art. See, e.g. J. Sambrook, supra. Such procedures may be employed in searching for persons with mutations in these receptors by the well-known techniques of restriction fragment length polymorphisms (RFLP), the procedures of which are described in U.S. Patent 4,666,828, issued May 19, 1987, the entire contents of which is herein incorporated by reference.

5 The proteins of this invention as well as fragments of these proteins may be used as antigens for the synthesis of antibodies. The term "antibody" as used herein describes antibodies, fragments of antibodies (such as, but not limited, to Fab, Fab', Fab², and Fv fragments), and chimeric, humanized, veneered, resurfaced, or CDR-grafted antibodies capable of binding antigens of a similar nature as the parent antibody molecule from which they are derived. The instant invention also encompasses single chain polypeptide binding molecules.

10 The term "antibody" as used herein is not limited by the manner in which the antibodies are produced, whether such production is in situ or not. The term "antibody" as used in this specification encompasses those antibodies produced by recombinant DNA technology means including, but not limited, to expression in bacteria, yeast, insect cell lines, or mammalian cell lines.

15 The production of antibodies, both monoclonal and polyclonal, in animals, especially mice, is well known in the art. See, e.g., C. Milstein, Handbook of Experimental Immunology, (Blackwell Scientific Pub., 1986); J. Goding, Monoclonal Antibodies: Principles and Practice, (Academic Press, 1983). For the production of monoclonal antibodies the basic process begins with injecting a mouse, or other suitable animal, with an immunogen. The mouse is subsequently sacrificed and cells taken from its spleen are fused with myeloma cells, resulting in a hybridoma that reproduces in vitro. The population of hybridomas is screened to isolate individual clones, each of which secretes a single antibody species, specific for the immunogen. The individual antibody species obtained in this way is each the product of a single B cell from the immune animal generated in response to a specific antigenic site, or epitope, recognized on the immunogenic substance.

20 Chimeric antibodies are described in U.S. Patent No. 4,816,567, which issued March 28, 1989 to S. Cabilly, et al. This reference discloses methods and vectors for the preparation of chimeric antibodies. The entire contents of U.S. Patent No. 4,816,567 are herein incorporated by reference. An alternative approach to production of genetically engineered antibodies is provided in U.S. Patent No. 4,816,397, which also issued March 28, 1989 to M. Boss, et al., the entire contents of which are herein incorporated by reference. The Boss patent teaches the simultaneous co-expression of the heavy and light chains of the antibody in the same host cell.

30 The approach of U.S. Patent 4,816,397 has been further refined as taught in European Patent Publication No. 0 239 400, which published September 30, 1987. The teachings of this European patent publication (Winter) are a preferred format for the genetic engineering of the reactive monoclonal antibodies of this invention. The Winter technology involves the replacement of complementarity determining regions (CDRs) of a human antibody with the CDRs of a murine monoclonal antibody thereby converting the specificity of the human antibody to the specificity of the murine antibody which was the source of the CDR regions. This "CDR grafting" technology affords a molecule containing minimal murine sequence and thus is less immunogenic.

35 Single chain antibody technology is yet another variety of genetically engineered antibody which is now well known in the art. See, e.g. R.E. Bird, et al., Science 242:423-426 (1988); PCT Publication No. WO 88/01649, which was published 10 March 1988. The single chain antibody technology involves joining the binding regions of heavy and light chains with a polypeptide sequence to generate a single polypeptide having the binding specificity of the antibody from which it was derived.

40 The aforementioned genetic engineering approaches provide the skilled artisan with numerous means to generate molecules which retain the binding characteristics of the parental antibody while affording a less immunogenic format.

45 These antibodies are used in diagnostics, therapeutics or in diagnostic/therapeutic combinations. By "diagnostics" as used herein is meant testing that is related to either the in vitro or in vivo diagnosis of disease states or biological status in mammals, preferably in humans. By "therapeutics" and "therapeutic/diagnostic combinations" as used herein is respectively meant the treatment or the diagnosis and treatment of disease states or biological status by the in vivo administration to mammals, preferably humans, of the antibodies of the present invention. The antibodies of the present invention are especially preferred in the diagnosis and/or treatment of conditions associated with an excess or deficiency of mGluR2 receptors.

50 In addition to being functional as direct therapeutic and diagnostic aids, the availability of a family of antibodies which are specific for the mGluR2 receptor enables the development of numerous assay systems for detecting agents which bind to this receptor. One such assay system comprises radiolabeling mGluR2 receptor-specific antibodies with a radionuclide such as ¹²⁵I and measuring displacement of the radiolabeled mGluR2 receptor-specific antibody from solid phase mGluR2 receptor in the presence of a potential antagonist.

55 Numerous other assay systems are also readily adaptable to detect agents which bind mGluR2 receptor. Examples of these aforementioned assay systems are discussed in Methods in Enzymology, (J. Langone, and H. Vunakis, eds. 1981), Vol. 73, Part B, the contents of which are herein incorporated by reference. Skilled artisans are directed to Section II of Methods in Enzymology, Vol. 73, Part B, supra, which discusses labeling of antibodies and antigens, and

Section IV, which discusses immunoassay methods.

In addition to the aforementioned antibodies specific for the mGluR2 receptor, this invention also provides antibodies which are specific for the hypervariable regions of the anti-mGluR2 receptor antibodies. Some such anti-idiotypic antibodies would resemble the original epitope, the mGluR2 receptor, and, therefore, would be useful in evaluating the effectiveness of compounds which are potential antagonists, agonists, or partial agonists of the mGluR2 receptor. See, e.g., Cleveland, et al., Nature (London), 305:56 (1983); Wasserman, et al., Proceedings of the National Academy of Sciences (USA), 79:4810 (1982).

In another embodiment, this invention encompasses pharmaceutical formulations for parenteral administration which contain, as the active ingredient, the anti-mGluR2 receptor antibodies described, supra. Such formulations are prepared by methods commonly used in pharmaceutical chemistry.

Products for parenteral administration are often formulated and distributed in solid, preferably freeze-dried form, for reconstitution immediately before use. Such formulations are useful compositions of the present invention. Their preparation is well understood by pharmaceutical chemists.

In general, these formulations comprise the active ingredient in combination with a mixture of inorganic salts, to confer isotonicity, as well as dispersing agents such as lactose, to allow the dried preparation to dissolve quickly upon reconstitution. Such formulations are reconstituted for use with highly purified water to a known concentration.

Alternatively, a water soluble form of the antibody can be dissolved in one of the commonly used intravenous fluids and administered by infusion. Such fluids include physiological saline, Ringer's solution or a 5% dextrose solution.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: ELI LILLY AND COMPANY
 (B) STREET: Lilly Corporate Center
 (C) CITY: Indianapolis
 (D) STATE: Indiana
 (E) COUNTRY: United States of America
 (F) ZIP: 46285
- (ii) TITLE OF INVENTION: EXCITATORY AMINO ACID RECEPTOR PROTEIN
 AND RELATED NUCLEIC ACID COMPOUNDS
- (iii) NUMBER OF SEQUENCES: 3
- (iv) CORRESPONDENCE ADDRESS:
 (A) ADDRESSEE: C. M. Hudson
 (B) STREET: Erl Wood Manor
 (C) CITY: Windlesham
 (D) STATE: Surrey
 (E) COUNTRY: United Kingdom
 (F) ZIP: GU20 6PH
- (v) COMPUTER READABLE FORM:
 (A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: Macintosh
 (C) OPERATING SYSTEM: Macintosh 7.0
 (D) SOFTWARE: Microsoft Word

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2619 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..2616

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

45	ATG GGA TCG CTG CTT GCG CTC CTG GCA CTG CTG CCG CTG TGG GGT GCT	48
	Met Gly Ser Leu Leu Ala Leu Leu Ala Leu Pro Leu Trp Gly Ala	
	1 5 10 15	
	GTG GCT GAG GGC CCA GCC AAG AAG GTG CTG ACC CTG GAG GGA GAC TTG	96
	Val Ala Glu Gly Pro Ala Lys Lys Val Leu Thr Leu Glu Gly Asp Leu	
50	20 25 30	
	GTG CTG GGT GGG CTG TTC CCA GTG CAC CAG AAG GGC GGC CCA GCA GAG	144
	Val Leu Gly Gly Leu Phe Pro Val His Gln Lys Gly Gly Pro Ala Glu	
	35 40 45	

EP 0 711 832 A2

5	GAC TGT GGT CCT GTC AAT GAG CAC CGT GGC ATC CAG CGC CTG GAG GCC Asp Cys Gly Pro Val Asn Glu His Arg Gly Ile Gln Arg Leu Glu Ala 50 55 60	192
10	ATG CTT TTT GCA CTG GAC CGC ATC AAC CGT GAC CCG CAC CTG CTG CCT Met Leu Phe Ala Leu Asp Arg Ile Asn Arg Asp Pro His Leu Leu Pro 65 70 75 80	240
15	GGC GTG CGC CTG GGT GCA CAC ATC CTC GAC AGT TGC TCC AAG GAC ACA Gly Val Arg Leu Gly Ala His Ile Leu Asp Ser Cys Ser Lys Asp Thr 85 90 95	288
20	CAT GCG CTG GAG CAG GCA CTG GAC TTT GTG CGT GCC TCA CTC AGC CGT His Ala Leu Glu Gln Ala Leu Asp Phe Val Arg Ala Ser Leu Ser Arg 100 105 110	336
25	GGT GCT GAT GGC TCA CGC CAC ATC TGC CCC GAC GGC TCT TAT GCG ACC Gly Ala Asp Gly Ser Arg His Ile Cys Pro Asp Gly Ser Tyr Ala Thr 115 120 125	384
30	CAT GGT GAT GCT CCC ACT GCC ATC ACT GGT GTT ATT GGC GGT TCC TAC His Gly Asp Ala Pro Thr Ala Ile Thr Gly Val Ile Gly Gly Ser Tyr 130 135 140	432
35	AGT GAT GTC TCC ATC CAG GTG GCC AAC CTC TTG AGG CTA TTT CAG ATC Ser Asp Val Ser Ile Gln Val Ala Asn Leu Leu Arg Leu Phe Gln Ile 145 150 155 160	480
40	CCA CAG ATT AGC TAC GCC TCT ACC AGT GCC AAG CTG AGT GAC AAG TCC Pro Gln Ile Ser Tyr Ala Ser Thr Ser Ala Lys Leu Ser Asp Lys Ser 165 170 175	528
45	CGC TAT GAC TAC TTT GCC CGC ACA GTG CCT CCT GAC TTC TTC CAA GCC Arg Tyr Asp Tyr Phe Ala Arg Thr Val Pro Pro Asp Phe Phe Gln Ala 180 185 190	576
50	AAG GCC ATG GCT GAG ATT CTC CGC TTC TTC AAC TGG ACC TAT GTG TCC Lys Ala Met Ala Glu Ile Leu Arg Phe Phe Asn Trp Thr Tyr Val Ser 195 200 205	624
55	ACT GTG GCG TCT GAG GGC GAC TAT GGC GAG ACA GGC ATT GAG GCC TTT Thr Val Ala Ser Glu Gly Asp Tyr Gly Glu Thr Gly Ile Glu Ala Phe 210 215 220	672
60	GAG CTA GAG GCT CGT GCC CGC AAC ATC TGT GTG GCC ACC TCG GAG AAA Glu Leu Glu Ala Arg Ala Arg Asn Ile Cys Val Ala Thr Ser Glu Lys 225 230 235 240	720
65	GTG GGC CGT GCC ATG AGC CGC GCG GCC TTT GAG GGT GTG GTG CGA GCC Val Gly Arg Ala Met Ser Arg Ala Ala Phe Glu Gly Val Val Arg Ala 245 250 255	768
70	CTG CTG CAG AAG CCC AGT GCC CGC GTG GCT GTC CTG TTC ACC CGT TCT Leu Leu Gln Lys Pro Ser Ala Arg Val Ala Val Leu Phe Thr Arg Ser 260 265 270	816
75	GAG GAT GCC CGG GAG CTG CTT GCT GCC AGC CAG CGC CTC AAT GCC AGC Glu Asp Ala Arg Glu Leu Leu Ala Ala Ser Gln Arg Leu Asn Ala Ser 275 280 285	864

EP 0 711 832 A2

	TTC	ACC	TGG	GTG	GCC	AGT	GAT	GGT	TGG	GGG	GCC	CTG	GAG	AGT	GTG	GTG	912
	Phe	Thr	Trp	Val	Ala	Ser	Asp	Gly	Trp	Gly	Ala	Leu	Glu	Ser	Val	Val	
		290					295					300					
5	GCA	GGC	AGT	GAG	GGG	GCT	GCT	GAG	GGT	GCT	ATC	ACC	ATC	GAG	CTG	GCC	960
	Ala	Gly	Ser	Glu	Gly	Ala	Ala	Glu	Gly	Ala	Ile	Thr	Ile	Glu	Leu	Ala	
	305				310					315						320	
10	TCC	TAC	CCC	ATC	AGT	GAC	TTT	GCC	TCC	TAC	TTC	CAG	AGC	CTG	GAC	CCT	1008
	Ser	Tyr	Pro	Ile	Ser	Asp	Phe	Ala	Ser	Tyr	Phe	Gln	Ser	Leu	Asp	Pro	
					325					330					335		
15	TGG	AAC	AAC	AGC	CGG	AAC	CCC	TGG	TTC	CGT	GAA	TTC	TGG	GAG	CAG	AGG	1056
	Trp	Asn	Asn	Ser	Arg	Asn	Pro	Trp	Phe	Arg	Glu	Phe	Trp	Glu	Gln	Arg	
				340					345					350			
	TTC	CGC	TGC	AGC	TTC	CGG	CAG	CGA	GAC	TGC	GCA	GCC	CAC	TCT	CTC	CGG	1104
	Phe	Arg	Cys	Ser	Phe	Arg	Gln	Arg	Asp	Cys	Ala	Ala	His	Ser	Leu	Arg	
			355					360					365				
20	GCT	GTG	CCC	TTT	GAG	CAG	GAG	TCC	AAG	ATC	ATG	TTT	GTG	GTC	AAT	GCA	1152
	Ala	Val	Pro	Phe	Glu	Gln	Glu	Ser	Lys	Ile	Met	Phe	Val	Val	Asn	Ala	
		370					375					380					
25	GTG	TAC	GCC	ATG	GCC	CAT	GCG	CTC	CAC	AAC	ATG	CAC	CGT	GCC	CTC	TGC	1200
	Val	Tyr	Ala	Met	Ala	His	Ala	Leu	His	Asn	Met	His	Arg	Ala	Leu	Cys	
	385					390					395					400	
	CCC	AAC	ACC	ACC	CGG	CTC	TGT	GAC	GCG	ATG	CGG	CCA	GTT	AAC	GGG	CGC	1248
	Pro	Asn	Thr	Thr	Arg	Leu	Cys	Asp	Ala	Met	Arg	Pro	Val	Asn	Gly	Arg	
					405					410					415		
30	CGC	CTC	TAC	AAG	GAC	TTT	GTG	CTC	AAC	GTC	AAG	TTT	GAT	GCC	CCC	TTT	1296
	Arg	Leu	Tyr	Lys	Asp	Phe	Val	Leu	Asn	Val	Lys	Phe	Asp	Ala	Pro	Phe	
				420					425					430			
35	CGC	CCA	GCT	GAC	ACC	CAC	AAT	GAG	GTC	CGC	TTT	GAC	CGC	TTT	GGT	GAT	1344
	Arg	Pro	Ala	Asp	Thr	His	Asn	Glu	Val	Arg	Phe	Asp	Arg	Phe	Gly	Asp	
			435					440					445				
40	GGT	ATT	GGC	CGC	TAC	AAC	ATC	TTC	ACC	TAT	CTG	CGT	GCA	GGC	AGT	GGG	1392
	Gly	Ile	Gly	Arg	Tyr	Asn	Ile	Phe	Thr	Tyr	Leu	Arg	Ala	Gly	Ser	Gly	
		450					455					460					
45	CGC	TAT	CGC	TAC	CAG	AAG	GTG	GGC	TAC	TGG	GCA	GAA	GGC	TTG	ACT	CTG	1440
	Arg	Tyr	Arg	Tyr	Gln	Lys	Val	Gly	Tyr	Trp	Ala	Glu	Gly	Leu	Thr	Leu	
	465					470					475					480	
50	GAC	ACC	AGC	CTC	ATC	CCA	TGG	GCC	TCA	CCC	TCA	GCC	GGC	CCC	CTG	CCC	1488
	Asp	Thr	Ser	Leu	Ile	Pro	Trp	Ala	Ser	Pro	Ser	Ala	Gly	Pro	Leu	Pro	
					485					490					495		
55	GCC	TCT	CGC	TGC	AGT	GAG	CCC	TGC	CTC	CAG	AAT	GAG	GTG	AAG	AGT	GTG	1536
	Ala	Ser	Arg	Cys	Ser	Glu	Pro	Cys	Leu	Gln	Asn	Glu	Val	Lys	Ser	Val	
				500				505					510				
	CAG	CCG	GGC	GAA	GTC	TGC	TGC	TGG	CTC	TGC	ATT	CCG	TGC	CAG	CCC	TAT	1584
	Gln	Pro	Gly	Glu	Val	Cys	Cys	Trp	Leu	Cys	Ile	Pro	Cys	Gln	Pro	Tyr	
			515					520					525				

EP 0 711 832 A2

		GAG	TAC	CGA	TTG	GAC	GAA	TTC	ACT	TGC	GCT	GAT	TGT	GGC	CTG	GGC	TAC	1632
		Glu	Tyr	Arg	Leu	Asp	Glu	Phe	Thr	Cys	Ala	Asp	Cys	Gly	Leu	Gly	Tyr	
		530						535					540					
5		TGG	CCC	AAT	GCC	AGC	CTG	ACT	GGC	TGC	TTC	GAA	CTG	CCC	CAG	GAG	TAC	1680
		Trp	Pro	Asn	Ala	Ser	Leu	Thr	Gly	Cys	Phe	Glu	Leu	Pro	Gln	Glu	Tyr	
		545					550					555					560	
10		ATC	CGC	TGG	GGC	GAT	GCC	TGG	GCT	GTG	GGA	CCT	GTC	ACC	ATC	GCC	TGC	1728
		Ile	Arg	Trp	Gly	Asp	Ala	Trp	Ala	Val	Gly	Pro	Val	Thr	Ile	Ala	Cys	
						565					570					575		
		CTC	GGT	GCC	CTG	GCC	ACC	CTC	TTT	GTG	CTG	GGT	GTC	TTT	GTG	CGG	CAC	1776
		Leu	Gly	Ala	Leu	Ala	Thr	Leu	Phe	Val	Leu	Gly	Val	Phe	Val	Arg	His	
15					580					585					590			
		AAT	GCC	ACA	CCA	GTG	GTC	AAG	GCC	TCA	GGT	CGG	GAG	CTC	TGC	TAC	ATC	1824
		Asn	Ala	Thr	Pro	Val	Val	Lys	Ala	Ser	Gly	Arg	Glu	Leu	Cys	Tyr	Ile	
					595				600					605				
20		CTG	CTG	GGT	GGT	GTC	TTC	CTC	TGC	TAC	TGC	ATG	ACC	TTC	ATC	TTC	ATT	1872
		Leu	Leu	Gly	Gly	Val	Phe	Leu	Cys	Tyr	Cys	Met	Thr	Phe	Ile	Phe	Ile	
			610					615					620					
		GCC	AAG	CCA	TCC	ACG	GGA	GTG	TGT	GCC	TTA	CGG	CGT	CTT	GGG	GTG	GGC	1920
25		Ala	Lys	Pro	Ser	Thr	Gly	Val	Cys	Ala	Leu	Arg	Arg	Leu	Gly	Val	Gly	
		625					630					635					640	
		ACT	GCC	TTC	TCT	GTC	TGC	TAC	TCA	GCC	CTG	CTC	ACC	AAG	ACC	AAC	CGC	1968
		Thr	Ala	Phe	Ser	Val	Cys	Tyr	Ser	Ala	Leu	Leu	Thr	Lys	Thr	Asn	Arg	
						645					650					655		
30		ATT	GCA	CGC	ATC	TTC	GGT	GGG	GCC	CGG	GAG	GGT	GCC	CAG	CGG	CCA	CGC	2016
		Ile	Ala	Arg	Ile	Phe	Gly	Gly	Ala	Arg	Glu	Gly	Ala	Gln	Arg	Pro	Arg	
						660				665					670			
35		TTC	ATC	AGT	CCT	GCC	TCA	CAG	GTG	GCC	ATC	TGC	CTG	GCA	CTT	ATC	TCG	2064
		Phe	Ile	Ser	Pro	Ala	Ser	Gln	Val	Ala	Ile	Cys	Leu	Ala	Leu	Ile	Ser	
				675					680					685				
		GGC	CAG	CTG	CTC	ATC	GTG	GTC	GCC	TGG	CTG	GTG	GTG	GAG	GCA	CCG	GGC	2112
		Gly	Gln	Leu	Leu	Ile	Val	Val	Ala	Trp	Leu	Val	Val	Glu	Ala	Pro	Gly	
40								690					700					
		ACA	GGC	AAG	GAG	ACA	GCC	CCC	GAA	CGG	CGG	GAG	GTG	GTG	ACA	CTG	CGC	2160
		Thr	Gly	Lys	Glu	Thr	Ala	Pro	Glu	Arg	Arg	Glu	Val	Val	Thr	Leu	Arg	
							705					710					720	
45		TGC	AAC	CAC	CGC	GAT	GCA	AGT	ATG	TTG	GGC	TCG	CTG	GCC	TAC	AAT	GTG	2208
		Cys	Asn	His	Arg	Asp	Ala	Ser	Met	Leu	Gly	Ser	Leu	Ala	Tyr	Asn	Val	
						725					730					735		
		CTC	CTC	ATC	GCG	CTC	TGC	ACG	CTT	TAT	GCC	TTC	AAG	ACT	CGC	AAG	TGC	2256
		Leu	Leu	Ile	Ala	Leu	Cys	Thr	Leu	Tyr	Ala	Phe	Lys	Thr	Arg	Lys	Cys	
50					740					745					750			
		CCC	GAA	AAC	TTC	AAC	GAG	GCC	AAG	TTC	ATT	GGC	TTC	ACC	ATG	TAC	ACC	2304
		Pro	Glu	Asn	Phe	Asn	Glu	Ala	Lys	Phe	Ile	Gly	Phe	Thr	Met	Tyr	Thr	
					755				760					765				
55																		

EP 0 711 832 A2

	ACC TGC ATC ATC TGG CTG GCA TTC TTG CCC ATC TTC TAT GTC ACC TCC	2352
	Thr Cys Ile Ile Trp Leu Ala Phe Leu Pro Ile Phe Tyr Val Thr Ser	
	770 775 780	
5	AGT GAC TAC CGG GTA CAG ACC ACC ACC ATG TGC GTG TCA GTC AGC CTC	2400
	Ser Asp Tyr Arg Val Gln Thr Thr Thr Met Cys Val Ser Val Ser Leu	
	785 790 795 800	
10	AGC GGC TCC GTG GTG CTT GGC TGC CTC TTT GCG CCC AAG CTG CAC ATC	2448
	Ser Gly Ser Val Val Leu Gly Cys Leu Phe Ala Pro Lys Leu His Ile	
	805 810 815	
15	ATC CTC TTC CAG CCG CAG AAG AAC GTG GTT AGC CAC CGG GCA CCC ACC	2496
	Ile Leu Phe Gln Pro Gln Lys Asn Val Val Ser His Arg Ala Pro Thr	
	820 825 830	
20	AGC CGC TTT GGC AGT GCT GCT GCC AGG GCC AGC TCC AGC CTT GGC CAA	2544
	Ser Arg Phe Gly Ser Ala Ala Ala Arg Ala Ser Ser Ser Leu Gly Gln	
	835 840 845	
25	GGG TCT GGC TCC CAG TTT GTC CCC ACT GTT TGC AAT GGC CGT GAG GTG	2592
	Gly Ser Gly Ser Gln Phe Val Pro Thr Val Cys Asn Gly Arg Glu Val	
	850 855 860	
30	GTG GAC TCG ACA ACG TCA TCG CTT TGA	2619
	Val Asp Ser Thr Thr Ser Ser Leu	
	865 870	

(2) INFORMATION FOR SEQ ID NO:2:

	(i) SEQUENCE CHARACTERISTICS:
30	(A) LENGTH: 872 amino acids
	(B) TYPE: amino acid
	(D) TOPOLOGY: linear
	(ii) MOLECULE TYPE: protein
35	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
	Met Gly Ser Leu Leu Ala Leu Leu Ala Leu Leu Pro Leu Trp Gly Ala
	1 5 10 15
40	Val Ala Glu Gly Pro Ala Lys Lys Val Leu Thr Leu Glu Gly Asp Leu
	20 25 30
	Val Leu Gly Gly Leu Phe Pro Val His Gln Lys Gly Gly Pro Ala Glu
	35 40 45
45	Asp Cys Gly Pro Val Asn Glu His Arg Gly Ile Gln Arg Leu Glu Ala
	50 55 60
	Met Leu Phe Ala Leu Asp Arg Ile Asn Arg Asp Pro His Leu Leu Pro
	65 70 75 80
50	Gly Val Arg Leu Gly Ala His Ile Leu Asp Ser Cys Ser Lys Asp Thr
	85 90 95
	His Ala Leu Glu Gln Ala Leu Asp Phe Val Arg Ala Ser Leu Ser Arg
	100 105 110
55	

EP 0 711 832 A2

	Gly	Ala	Asp	Gly	Ser	Arg	His	Ile	Cys	Pro	Asp	Gly	Ser	Tyr	Ala	Thr	
			115					120					125				
5	His	Gly	Asp	Ala	Pro	Thr	Ala	Ile	Thr	Gly	Val	Ile	Gly	Gly	Ser	Tyr	
		130					135					140					
	Ser	Asp	Val	Ser	Ile	Gln	Val	Ala	Asn	Leu	Leu	Arg	Leu	Phe	Gln	Ile	
	145					150					155					160	
10	Pro	Gln	Ile	Ser	Tyr	Ala	Ser	Thr	Ser	Ala	Lys	Leu	Ser	Asp	Lys	Ser	
					165					170					175		
	Arg	Tyr	Asp	Tyr	Phe	Ala	Arg	Thr	Val	Pro	Pro	Asp	Phe	Phe	Gln	Ala	
				180					185						190		
15	Lys	Ala	Met	Ala	Glu	Ile	Leu	Arg	Phe	Phe	Asn	Trp	Thr	Tyr	Val	Ser	
			195					200					205				
	Thr	Val	Ala	Ser	Glu	Gly	Asp	Tyr	Gly	Glu	Thr	Gly	Ile	Glu	Ala	Phe	
20		210					215					220					
	Glu	Leu	Glu	Ala	Arg	Ala	Arg	Asn	Ile	Cys	Val	Ala	Thr	Ser	Glu	Lys	
	225					230					235					240	
	Val	Gly	Arg	Ala	Met	Ser	Arg	Ala	Ala	Phe	Glu	Gly	Val	Val	Arg	Ala	
25					245					250					255		
	Leu	Leu	Gln	Lys	Pro	Ser	Ala	Arg	Val	Ala	Val	Leu	Phe	Thr	Arg	Ser	
				260					265					270			
	Glu	Asp	Ala	Arg	Glu	Leu	Leu	Ala	Ala	Ser	Gln	Arg	Leu	Asn	Ala	Ser	
30			275					280					285				
	Phe	Thr	Trp	Val	Ala	Ser	Asp	Gly	Trp	Gly	Ala	Leu	Glu	Ser	Val	Val	
		290					295					300					
	Ala	Gly	Ser	Glu	Gly	Ala	Ala	Glu	Gly	Ala	Ile	Thr	Ile	Glu	Leu	Ala	
35		305				310					315					320	
	Ser	Tyr	Pro	Ile	Ser	Asp	Phe	Ala	Ser	Tyr	Phe	Gln	Ser	Leu	Asp	Pro	
					325					330					335		
40	Trp	Asn	Asn	Ser	Arg	Asn	Pro	Trp	Phe	Arg	Glu	Phe	Trp	Glu	Gln	Arg	
				340					345					350			
	Phe	Arg	Cys	Ser	Phe	Arg	Gln	Arg	Asp	Cys	Ala	Ala	His	Ser	Leu	Arg	
			355					360					365				
45	Ala	Val	Pro	Phe	Glu	Gln	Glu	Ser	Lys	Ile	Met	Phe	Val	Val	Asn	Ala	
		370					375					380					
	Val	Tyr	Ala	Met	Ala	His	Ala	Leu	His	Asn	Met	His	Arg	Ala	Leu	Cys	
	385					390					395					400	
50	Pro	Asn	Thr	Thr	Arg	Leu	Cys	Asp	Ala	Met	Arg	Pro	Val	Asn	Gly	Arg	
					405					410					415		
	Arg	Leu	Tyr	Lys	Asp	Phe	Val	Leu	Asn	Val	Lys	Phe	Asp	Ala	Pro	Phe	
55				420					425					430			

EP 0 711 832 A2

	Arg	Pro	Ala	Asp	Thr	His	Asn	Glu	Val	Arg	Phe	Asp	Arg	Phe	Gly	Asp	
			435					440					445				
5	Gly	Ile	Gly	Arg	Tyr	Asn	Ile	Phe	Thr	Tyr	Leu	Arg	Ala	Gly	Ser	Gly	
		450					455					460					
	Arg	Tyr	Arg	Tyr	Gln	Lys	Val	Gly	Tyr	Trp	Ala	Glu	Gly	Leu	Thr	Leu	
	465					470					475					480	
10	Asp	Thr	Ser	Leu	Ile	Pro	Trp	Ala	Ser	Pro	Ser	Ala	Gly	Pro	Leu	Pro	
					485					490					495		
	Ala	Ser	Arg	Cys	Ser	Glu	Pro	Cys	Leu	Gln	Asn	Glu	Val	Lys	Ser	Val	
				500					505					510			
15	Gln	Pro	Gly	Glu	Val	Cys	Cys	Trp	Leu	Cys	Ile	Pro	Cys	Gln	Pro	Tyr	
			515					520					525				
	Glu	Tyr	Arg	Leu	Asp	Glu	Phe	Thr	Cys	Ala	Asp	Cys	Gly	Leu	Gly	Tyr	
		530					535					540					
20	Trp	Pro	Asn	Ala	Ser	Leu	Thr	Gly	Cys	Phe	Glu	Leu	Pro	Gln	Glu	Tyr	
	545					550					555					560	
	Ile	Arg	Trp	Gly	Asp	Ala	Trp	Ala	Val	Gly	Pro	Val	Thr	Ile	Ala	Cys	
25					565					570					575		
	Leu	Gly	Ala	Leu	Ala	Thr	Leu	Phe	Val	Leu	Gly	Val	Phe	Val	Arg	His	
				580				585						590			
	Asn	Ala	Thr	Pro	Val	Val	Lys	Ala	Ser	Gly	Arg	Glu	Leu	Cys	Tyr	Ile	
30			595					600					605				
	Leu	Leu	Gly	Gly	Val	Phe	Leu	Cys	Tyr	Cys	Met	Thr	Phe	Ile	Phe	Ile	
		610					615					620					
35	Ala	Lys	Pro	Ser	Thr	Gly	Val	Cys	Ala	Leu	Arg	Arg	Leu	Gly	Val	Gly	
	625					630					635					640	
	Thr	Ala	Phe	Ser	Val	Cys	Tyr	Ser	Ala	Leu	Leu	Thr	Lys	Thr	Asn	Arg	
					645					650					655		
40	Ile	Ala	Arg	Ile	Phe	Gly	Gly	Ala	Arg	Glu	Gly	Ala	Gln	Arg	Pro	Arg	
				660					665					670			
	Phe	Ile	Ser	Pro	Ala	Ser	Gln	Val	Ala	Ile	Cys	Leu	Ala	Leu	Ile	Ser	
			675				680						685				
45	Gly	Gln	Leu	Leu	Ile	Val	Val	Ala	Trp	Leu	Val	Val	Glu	Ala	Pro	Gly	
		690					695						700				
	Thr	Gly	Lys	Glu	Thr	Ala	Pro	Glu	Arg	Arg	Glu	Val	Val	Thr	Leu	Arg	
	705					710					715					720	
50	Cys	Asn	His	Arg	Asp	Ala	Ser	Met	Leu	Gly	Ser	Leu	Ala	Tyr	Asn	Val	
					725					730					735		
	Leu	Leu	Ile	Ala	Leu	Cys	Thr	Leu	Tyr	Ala	Phe	Lys	Thr	Arg	Lys	Cys	
55				740					745					750			

EP 0 711 832 A2

Pro Glu Asn Phe Asn Glu Ala Lys Phe Ile Gly Phe Thr Met Tyr Thr
755 760 765

5 Thr Cys Ile Ile Trp Leu Ala Phe Leu Pro Ile Phe Tyr Val Thr Ser
770 775 780

Ser Asp Tyr Arg Val Gln Thr Thr Thr Met Cys Val Ser Val Ser Leu
785 790 795 800

10 Ser Gly Ser Val Val Leu Gly Cys Leu Phe Ala Pro Lys Leu His Ile
805 810 815

Ile Leu Phe Gln Pro Gln Lys Asn Val Val Ser His Arg Ala Pro Thr
820 825 830

15 Ser Arg Phe Gly Ser Ala Ala Ala Arg Ala Ser Ser Ser Leu Gly Gln
835 840 845

Gly Ser Gly Ser Gln Phe Val Pro Thr Val Cys Asn Gly Arg Glu Val
850 855 860

20 Val Asp Ser Thr Thr Ser Ser Leu
865 870

(2) INFORMATION FOR SEQ ID NO:3:

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2619 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: mRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

35 AUGGGAUCGC UGCUUGCGCU CCUGGCACUG CUGCCGCUGU GGGGUGCUGU GGCUGAGGGC 60
CCAGCCAAGA AGGUGCUGAC CCUGGAGGGA GACUUGGUGC UGGGUGGGCU GUUCCCAGUG 120
CACCAGAAGG GCGGCCCAGC AGAGGACUGU GGUCCUGUCA AUGAGCACCG UGGCAUCCAG 180
40 CGCCUGGAGG CCAUGCUUUU UGCACUGGAC CGCAUCAACC GUGACCCGCA CCUGCUGCCU 240
GGCGUGCGCC UGGGUGCACA CAUCCUCGAC AGUUGCUGCA AGGACACACA UGCGCUGGAG 300
CAGGCACUGG ACUUGUGCG UGCCUCACUC AGCCGUGGUG CUGAUGGCUC ACGCCACAUC 360
45 UGCCCCGACG GCUCUUAUGC GACCCAUGGU GAUGCUGCCA CUGCCAUCAC UGGUGUUUUU 420
GGCGGUUCCU ACAGUGAUGU CUCCAUCCAG GUGGCCAACC UCUUGAGGCU AUUUCAGAUC 480
CCACAGAUUA GCUACGCCUC UACCAGUGCC AAGCUGAGUG ACAAGUCCCG CUAUGACUAC 540
50 UUUGCCCGCA CAGUGCCUCC UGACUUCUUC CAAGCCAAGG CCAUGGCUGA GAUUCUCCGC 600
UUCUUAACU GGACCUAUGU GUCCACUGUG GCGUCUGAGG GCGACUAUGG CGAGACAGGC 660
AUUGAGGCCU UUGAGCUAGA GGCUCGUGCC CGCAACAUCU GUGUGGCCAC CUCGGAGAAA 720
55 GUGGGCCGUG CCAUGAGCCG CGCGGCCUUU GAGGGUGUGG UGCGAGCCCU GCUGCAGAAG 780

EP 0 711 832 A2

	CCCAGUGCCC GCGUGGCUGU CCUGUUCACC CGUUCUGAGG AUGCCCGGGA GCUGCUUGCU	840
	GCCAGCCAGC GCCUCAAUGC CAGCUUCACC UGGGUGGCCA GUGAUGGUUG GGGGGCCUG	900
5	GAGAGUGUGG UGGCAGGCAG UGAGGGGGCU GCUGAGGGUG CUAUCACCAU CGAGCUGGCC	960
	UCCUACCCCA UCAGUGACUU UGCCUCCUAC UUCCAGAGCC UGGACCCUUG GAACAACAGC	1020
	CGGAACCCCU GGUUCCGUGA AUUCUGGGAG CAGAGGUUCC GCUGCAGCUU CCGGCAGCGA	1080
10	GACUGCGCAG CCCACUCUCU CCGGGCUGUG CCCUUGAGC AGGAGUCCAA GAUCAUGUUU	1140
	GUGGUCAAUG CAGUGUACGC CAUGGGCCCAU GCGCUCCACA ACAUGCACCG UGCCCUCUGC	1200
	CCCAACACCA CCCGGCUCUG UGACGCGAUG CGGCCAGUUA ACGGGCGCCG CCUCUACAAG	1260
15	GACUUUGUGC UCAACGUCAA GUUUGAUGCC CCCUUUGGCC CAGCUGACAC CCACAAUGAG	1320
	GUCCGCUUUG ACCGCUUUGG UGAUGGUUUU GGCCGCUACA ACAUCUUCAC CUAUCUGCGU	1380
	GCAGGCAGUG GGCGCUAUCG CUACCAGAAG GUGGGCUACU GGGCAGAAGG CUUGACUCUG	1440
20	GACACCAGCC UCAUCCCAUG GGCCUACCC UCAGCCGGCC CCCUGCCCGC CUCUCGCUGC	1500
	AGUGAGCCCU GCCUCCAGAA UGAGGUGAAG AGUGUGCAGC CGGGCGAAGU CUGCUGCUGG	1560
	CUCUGCAUUC CGUGCCAGCC CUAUGAGUAC CGAUUGGACG AAUUCACUUG CGCUGAUUGU	1620
25	GGCCUGGGCU ACUGGGCCAA UGCCAGCCUG ACUGGCUGCU UCGAACUGCC CCAGGAGUAC	1680
	AUCCGCUGGG GCGAUGCCUG GGCUGUGGGA CCUGUCACCA UCGCCUGCCU CGGUGCCCUG	1740
	GCCACCCUCU UUGUGCUGGG UGUUUUUGUG CGGCACAAUG CCACACCAGU GGUCAAGGCC	1800
30	UCAGGUCGGG AGCUCUGCUA CAUCCUGCUG GGUGGUGUCU UCCUCUGCUA CUGCAUGACC	1860
	UUCAUCUUA UUGCCAAGCC AUCCACGGGA GUGUGUGCCU UACGGCGUCU UGGGGUGGGC	1920
35	ACUGCCUUCU CUGUCUGCUA CUCAGCCCUG CUCACCAAGA CCAACCGCAU UGCACGCAUC	1980
	UUCGGUGGGG CCCGGGAGGG UGCCAGCGG CCACGCUUCA UCAGUCCUGC CUCACAGGUG	2040
	GCCAUCUGCC UGGCACUUAU CUCGGGCCAG CUGCUCaucG UGGUCGCCUG GCUGGUGGUG	2100
40	GAGGCACCGG GCACAGGCAA GGAGACAGCC CCCGAACGGC GGGAGGUGGU GACACUGCGC	2160
	UGCAACCACC GCGAUGCAAG UAUGUUGGGC UCGCUGGCCU ACA AUGUGCU CCUCAUCGCG	2220
	CUCUGCACGC UUU AUGCCUU CAAGACUCGC AAGUGCCCCG AAAACUCAA CGAGGCCAAG	2280
45	UUCAUUGGCU UCACCAUGUA CACCACCUGC AUCAUCUGGC UGGCAUUCUU GCCCAUCUUC	2340
	UAUGUACCU CCAGUGACUA CCGGGUACAG ACCACCACCA UGUGCGUGUC AGUCAGCCUC	2400
	AGCGGCUCCG UGGUGCUUGG CUGCCUCUUU GCGCCCAAGC UGCACAUCAU CCUCUUCAG	2460
50	CCGCAGAAGA ACGUGGUUAG CCACCGGGCA CCCACCAGCC GCUUUGGCAG UGCUGCUGCC	2520
	AGGGCCAGCU CCAGCCUUGG CCAAGGGUCU GGCUCCAGU UUGUCCCCAC UGUUUGCAAU	2580
55	GGCCGUGAGG UGGUGGACUC GACAACGUCA UCGCUUUGA	2619

Claims

1. An isolated amino acid compound functional as a human metabotropic glutamate receptor which comprises the amino acid sequence

5
Met Gly Ser Leu Leu Ala Leu Leu Ala Leu Leu Pro Leu Trp Gly Ala
1 5 10 15

10 Val Ala Glu Gly Pro Ala Lys Lys Val Leu Thr Leu Glu Gly Asp Leu
20 25 30

Val Leu Gly Gly Leu Phe Pro Val His Gln Lys Gly Gly Pro Ala Glu
35 40 45

15 Asp Cys Gly Pro Val Asn Glu His Arg Gly Ile Gln Arg Leu Glu Ala
50 55 60

Met Leu Phe Ala Leu Asp Arg Ile Asn Arg Asp Pro His Leu Leu Pro
65 70 75 80

20 Gly Val Arg Leu Gly Ala His Ile Leu Asp Ser Cys Ser Lys Asp Thr
85 90 95

25 His Ala Leu Glu Gln Ala Leu Asp Phe Val Arg Ala Ser Leu Ser Arg
100 105 110

Gly Ala Asp Gly Ser Arg His Ile Cys Pro Asp Gly Ser Tyr Ala Thr
115 120 125

30 His Gly Asp Ala Pro Thr Ala Ile Thr Gly Val Ile Gly Gly Ser Tyr
130 135 140

Ser Asp Val Ser Ile Gln Val Ala Asn Leu Leu Arg Leu Phe Gln Ile
145 150 155 160

35 Pro Gln Ile Ser Tyr Ala Ser Thr Ser Ala Lys Leu Ser Asp Lys Ser
165 170 175

40 Arg Tyr Asp Tyr Phe Ala Arg Thr Val Pro Pro Asp Phe Phe Gln Ala
180 185 190

Lys Ala Met Ala Glu Ile Leu Arg Phe Phe Asn Trp Thr Tyr Val Ser
195 200 205

45 Thr Val Ala Ser Glu Gly Asp Tyr Gly Glu Thr Gly Ile Glu Ala Phe
210 215 220

Glu Leu Glu Ala Arg Ala Arg Asn Ile Cys Val Ala Thr Ser Glu Lys
225 230 235 240

50

55

EP 0 711 832 A2

Val Gly Arg Ala Met Ser Arg Ala Ala Phe Glu Gly Val Val Arg Ala
245 250 255

5 Leu Leu Gln Lys Pro Ser Ala Arg Val Ala Val Leu Phe Thr Arg Ser
260 265 270

Glu Asp Ala Arg Glu Leu Leu Ala Ala Ser Gln Arg Leu Asn Ala Ser
275 280 285

10 Phe Thr Trp Val Ala Ser Asp Gly Trp Gly Ala Leu Glu Ser Val Val
290 295 300

Ala Gly Ser Glu Gly Ala Ala Glu Gly Ala Ile Thr Ile Glu Leu Ala
305 310 315 320

Ser Tyr Pro Ile Ser Asp Phe Ala Ser Tyr Phe Gln Ser Leu Asp Pro
325 330 335

20 Trp Asn Asn Ser Arg Asn Pro Trp Phe Arg Glu Phe Trp Glu Gln Arg
340 345 350

Phe Arg Cys Ser Phe Arg Gln Arg Asp Cys Ala Ala His Ser Leu Arg
355 360 365

25 Ala Val Pro Phe Glu Gln Glu Ser Lys Ile Met Phe Val Val Asn Ala
370 375 380

Val Tyr Ala Met Ala His Ala Leu His Asn Met His Arg Ala Leu Cys
385 390 395 400

Pro Asn Thr Thr Arg Leu Cys Asp Ala Met Arg Pro Val Asn Gly Arg
405 410 415

35 Arg Leu Tyr Lys Asp Phe Val Leu Asn Val Lys Phe Asp Ala Pro Phe
420 425 430

Arg Pro Ala Asp Thr His Asn Glu Val Arg Phe Asp Arg Phe Gly Asp
435 440 445

40 Gly Ile Gly Arg Tyr Asn Ile Phe Thr Tyr Leu Arg Ala Gly Ser Gly
450 455 460

Arg Tyr Arg Tyr Gln Lys Val Gly Tyr Trp Ala Glu Gly Leu Thr Leu
465 470 475 480

Asp Thr Ser Leu Ile Pro Trp Ala Ser Pro Ser Ala Gly Pro Leu Pro
485 490 495

50 Ala Ser Arg Cys Ser Glu Pro Cys Leu Gln Asn Glu Val Lys Ser Val
500 505 510

Gln Pro Gly Glu Val Cys Cys Trp Leu Cys Ile Pro Cys Gln Pro Tyr
515 520 525

55

EP 0 711 832 A2

5 Glu Tyr Arg Leu Asp Glu Phe Thr Cys Ala Asp Cys Gly Leu Gly Tyr
 530 535 540

 Trp Pro Asn Ala Ser Leu Thr Gly Cys Phe Glu Leu Pro Gln Glu Tyr
 545 550 555 560

10 Ile Arg Trp Gly Asp Ala Trp Ala Val Gly Pro Val Thr Ile Ala Cys
 565 570 575

 Leu Gly Ala Leu Ala Thr Leu Phe Val Leu Gly Val Phe Val Arg His
 580 585 590

15 Asn Ala Thr Pro Val Val Lys Ala Ser Gly Arg Glu Leu Cys Tyr Ile
 595 600 605

 Leu Leu Gly Gly Val Phe Leu Cys Tyr Cys Met Thr Phe Ile Phe Ile
 610 615 620

20 Ala Lys Pro Ser Thr Gly Val Cys Ala Leu Arg Arg Leu Gly Val Gly
 625 630 635 640

 Thr Ala Phe Ser Val Cys Tyr Ser Ala Leu Leu Thr Lys Thr Asn Arg
 645 650 655

25 Ile Ala Arg Ile Phe Gly Gly Ala Arg Glu Gly Ala Gln Arg Pro Arg
 660 665 670

30 Phe Ile Ser Pro Ala Ser Gln Val Ala Ile Cys Leu Ala Leu Ile Ser
 675 680 685

 Gly Gln Leu Leu Ile Val Val Ala Trp Leu Val Val Glu Ala Pro Gly
 690 695 700

35 Thr Gly Lys Glu Thr Ala Pro Glu Arg Arg Glu Val Val Thr Leu Arg
 705 710 715 720

40 Cys Asn His Arg Asp Ala Ser Met Leu Gly Ser Leu Ala Tyr Asn Val
 725 730 735

 Leu Leu Ile Ala Leu Cys Thr Leu Tyr Ala Phe Lys Thr Arg Lys Cys
 740 745 750

45 Pro Glu Asn Phe Asn Glu Ala Lys Phe Ile Gly Phe Thr Met Tyr Thr
 755 760 765

50 Thr Cys Ile Ile Trp Leu Ala Phe Leu Pro Ile Phe Tyr Val Thr Ser
 770 775 780

 Ser Asp Tyr Arg Val Gln Thr Thr Thr Met Cys Val Ser Val Ser Leu
 785 790 795 800

55

Ser Gly Ser Val Val Leu Gly Cys Leu Phe Ala Pro Lys Leu His Ile
 805 810 815
 5 Ile Leu Phe Gln Pro Gln Lys Asn Val Val Ser His Arg Ala Pro Thr
 820 825 830
 Ser Arg Phe Gly Ser Ala Ala Ala Arg Ala Ser Ser Ser Leu Gly Gln
 835 840 845
 10 Gly Ser Gly Ser Gln Phe Val Pro Thr Val Cys Asn Gly Arg Glu Val
 850 855 860
 Val Asp Ser Thr Thr Ser Ser Leu
 865 870
 15

identified herein as SEQ ID NO:2, or a functional equivalent thereof, or a fragment of at least 6 continuous amino acids thereof.

- 20 2. A nucleic acid compound encoding an amino acid compound as claimed in **Claim 1**.
3. A composition comprising an isolated nucleic acid compound containing a sequence encoding a human glutamate receptor or fragment thereof as claimed in **Claim 2**, wherein said sequence encoding a human glutamate receptor or fragment thereof is selected from the group consisting of:

25 (a) ATGGGATCGC TGCTTGCGCT CCTGGCACTG CTGCCGCTGT GGGGTGCTGT
 GGCTGAGGGC CCAGCCAAGA AGGTGCTGAC CCTGGAGGGA GACTTGGTGC
 30 TGGGTGGGCT GTTCCCAGTG CACCAGAAGG GCGGCCCAGC AGAGGACTGT
 GGTCTGTCA ATGAGCACCG TGGCATCCAG CGCCTGGAGG CCATGCTTTT
 TGCACTGGAC CGCATCAACC GTGACCCGCA CCTGCTGCCT GGCGTGCGCC
 35 TGGGTGCACA CATCCTCGAC AGTTGCTCCA AGGACACACA TCGCTGGAG
 CAGGCACTGG ACTTTGTGCG TGCCTCACTC AGCCGTGGTG CTGATGGCTC
 ACGCCACATC TGCCCCGACG GCTCTTATGC GACCCATGGT GATGCTCCCA
 40 CTGCCATCAC TGGTGTTATT GCGGGTTCCT ACAGTGATGT CTCCATCCAG
 GTGGCCAACC TCTTGAGGCT ATTCAGATC CCACAGATTA GCTACGCCTC
 45

50

55

TACCAAGTGCC AAGCTGAGTG ACAAGTCCCG CTATGACTAC TTTGCCCCGCA
 5 CAGTGCCTCC TGACTTCTTC CAAGCCAAAG CCATGGGCTGA GATTCTCCGC
 TTCTTCAACT GGACCTATGT GTCCACTGTG GCGTCTGAGG GCGACTATGG
 CGAGACAGGC ATTGAGGCCT TTGAGCTAGA GGCTCGTGCC CGCAACATCT
 10 GTGTGGCCAC CTCGGAGAAA GTGGGCCGTG CCATGAGCCG CGCGGCCTTT
 GAGGGTGTGG TCGGAGCCCT GCTGCAGAAG CCCAGTGCCC GCGTGGCTGT
 CCTGTTTACC CGTTCTGAGG ATGCCCCGGA GCTGCTTGCT GCCAGCCAGC
 15 GCCTCAATGC CAGCTTCACC TGGGTGGCCA GTGATGGTTG GGGGGCCCTG
 GAGAGTGTGG TGGCAGGCAG TGAGGGGGCT GCTGAGGGTG CTATCACCAT
 CGAGCTGGCC TCCTACCCCA TCAGTGACTT TGCCTCCTAC TTCCAGAGCC
 20 TGGACCCTTG GAACAACAGC CGGAACCCCT GGTTCCGTGA ATTCTGGGAG
 CAGAGGTTCC GCTGCAGCTT CCGGCAGCGA GACTGCGCAG CCCACTCTCT
 CCGGGCTGTG CCCTTTGAGC AGGAGTCCAA GATCATGTTT GTGGTCAATG
 25 CAGTGTACGC CATGGCCCAT GCGCTCCACA ACATGCACCG TGCCCTCTGC
 CCCAACACCA CCGGCTCTG TGACGCGATG CGGCCAGTTA ACGGGCGCCG
 30 CCTCTACAAG GACTTTGTGC TCAACGTCAA GTTTGATGCC CCCTTTCGCC
 CAGCTGACAC CCACAATGAG GTCCGCTTTG ACCGCTTTGG TGATGGTATT
 GGCCGCTACA ACATCTTCAC CTATCTGCGT GCAGGCAGTG GGCGCTATCG
 35 CTACCAGAAG GTGGGCTACT GGGCAGAAGG CTTGACTCTG GACACCAGCC
 TCATCCCATG GGCTTCACCC TCAGCCGGCC CCCTGCCCCG CTCTCGCTGC
 AGTGAGCCCT GCCTCCAGAA TGAGGTGAAG AGTGTGCAGC CGGGCGAAGT
 40 CTGCTGCTGG CTCTGCATTG CGTGCCAGCC CTATGAGTAC CGATTGGACG
 AATTCACTTG CGCTGATTGT GGCCTGGGCT ACTGGCCCAA TGCCAGCCTG
 45 ACTGGCTGCT TCGAACTGCC CCAGGAGTAC ATCCGCTGGG GCGATGCCTG
 GGCTGTGGGA CCTGTCACCA TCGCCTGCCT CGGTGCCCTG GCCACCCTCT
 TTGTGCTGGG TGTCTTTGTG CGGCACAATG CCACACCAGT GGTCAAGGCC
 50 TCAGGTGCGG AGCTCTGCTA CATCCTGCTG GGTGGTGTCT TCCTCTGCTA
 CTGCATGACC TTCATCTTCA TTGCCAAGCC ATCCACGGGA GTGTGTGCCT
 55 TACGGCGTCT TGGGGTGGGC ACTGCCTTCT CTGTCTGCTA CTCAGCCCTG

5

10

15

20

25

CTCACCAAGA CCAACCGCAT TGCACGCATC TTCGGTGGGG CCCGGGAGGG
 TGCCCAGCGG CCACGCTTCA TCAGTCCTGC CTCACAGGTG GCCATCTGCC
 TGGCACTTAT CTCGGGCCAG CTGCTCATCG TGGTCGCCTG GCTGGTGGTG
 GAGGCACCGG GCACAGGCAA GGAGACAGCC CCCGAACGGC GGGAGGTGGT
 GACACTGCGC TGCAACCACC GCGATGCAAG TATGTTGGGC TCGCTGGCCT
 ACAATGTGCT CCTCATCGCG CTCTGCACGC TTTATGCCTT CAAGACTCGC
 AAGTGCCCCG AAAACTTCAA CGAGGCCAAG TTCATTGGCT TCACCATGTA
 CACCACCTGC ATCATCTGGC TGGCATTTCTT GCCCATCTTC TATGTCACCT
 CCAGTGACTA CCGGGTACAG ACCACCACCA TGTGCGTGTC AGTCAGCCTC
 AGCGGCTCCG TGGTGCTTGG CTGCCTCTTT GCGCCCAAGC TGCACATCAT
 CCTCTTCCAG CCGCAGAAGA ACGTGGTTAG CCACCGGGCA CCCACCAGCC
 GCTTTGGCAG TGCTGCTGCC AGGGCCAGCT CCAGCCTTGG CCAAGGGTCT
 GGCTCCCAGT TTGTCCCCAC TGTTTGCAAT GGCCGTGAGG TGGTGGACTC
 GACAACGTCA TCGCTTTGA

identified herein as SEQ ID NO:1;

30

35

40

45

50

55

(b) AUGGGAUCGC UGCUUGCGCU CCUGGCACUG CUGCCGCUGU GGGGUGCUGU
 GGCUGAGGGC CCAGCCAAGA AGGUGCUGAC CCUGGAGGGA GACUUGGUGC
 UGGGUGGGCU GUUCCCAGUG CACCAGAAGG GCGGCCCAGC AGAGGACUGU
 GGUCCUGUCA AUGAGCACCG UGGCAUCCAG CGCCUGGAGG CCAUGCUUUU
 UGCACUGGAC CGCAUCAACC GUGACCCGCA CCUGCUGCCU GGCUGCGGCC
 UGGGUGCACA CAUCCUCGAC AGUUGCUGCA AGGACACACA UGCGCUGGAG
 CAGGCACUGG ACUUGUGCG UGCCUCACUC AGCCGUGGUG CUGAUGGCUC
 ACGCCACAUC UGCCCCGACG GCUCUUAUGC GACCCAUGGU GAUGCUGCCA
 CUGCCAUCAC UGGUGUUAUU GCGGGUCCU ACAGUGAUGU CUCCAUCCAG
 GUGGCCAACC UCUUGAGGCU AUUUCAGAUC CCACAGAUUA GCUACGCCUC
 UACCAGUGCC AAGCUGAGUG ACAAGUCCG CUAUGACUAC UUUGCCCGCA
 CAGUGCCUCC UGACUUCUUC CAAGCCAAGG CCAUGGCUGA GAUUCUCCGC
 UUCUUAACU GGACCUAUGU GUCCACUGUG GCGUCUGAGG GCGACUAUGG
 CGAGACAGGC AUUGAGGCCU UUGAGCUAGA GGCUCGUGCC CGCAACAUCU

GUGUGGCCAC CUCGGAGAAA GUGGGCCGUG CCAUGAGCCG CGCGGCCUUU
 GAGGGUGUGG UGCGAGCCCU GCUGCAGAAG CCCAGUGCCC GCGUGGCUGU
 5 CCUGUUCACC CGUUCUGAGG AUGCCCGGGA GCUGCUUGCU GCCAGCCAGC
 GCCUCA AUGC CAGCUUCACC UGGGUGGCCA GUGAUGGUUG GGGGGCCCCUG
 10 GAGAGUGUGG UGGCAGGCAG UGAGGGGGCU GCUGAGGGUG CUAUCACCAU
 CGAGCUGGCC UCCUACCCCA UCAGUGACUU UGCCUCCUAC UUCAGAGCC
 UGGACCCUUG GAACAACAGC CGGAACCCCU GGUUCCGUGA AUUCUGGGAG
 15 CAGAGGUUCC GCUGCAGCUU CCGGCAGCGA GACUGCGCAG CCCACUCUCU
 CCGGGCUGUG CCCUUGAGC AGGAGUCCAA GAUCAUGUUU GUGGUCAAUG
 CAGUGUACGC CAUGGCCCAU GCGCUCCACA ACAUGCACCG UGCCCUCUGC
 20 CCCAACACCA CCCGGCUCUG UGACGCGAUG CGGCCAGUUA ACGGGCGCCC
 CCUCUACAAG GACUUUGUGC UCAACGUCAA GUUUGAUGCC CCCUUUCGCC
 25 CAGCUGACAC CCACAAUGAG GUCCGCUUUG ACCGCUUUGG UGAUGGUAAU
 GGCCGCUACA ACAUCUUCAC CUAUCUGCGU GCAGGCAGUG GGCGCUAUCG
 CUACCAGAAG GUGGGCUACU GGGCAGAAGG CUUGACUCUG GACACCAGCC
 30 UCAUCCCAUG GGCCUCACCC UCAGCCGGCC CCCUGCCCGC CUCUCGCUGC
 AGUGAGCCCU GCCUCCAGAA UGAGGUGAAG AGUGUGCAGC CGGGCGAAGU
 CUGCUGCUGG CUCUGCAUUC CGUGCCAGCC CUAUGAGUAC CGAUUGGACG
 35 AAUUCACUUG CGCUGAUUGU GGCCUGGGCU ACUGGCCCAA UGCCAGCCUG
 ACUGGCUGCU UCGAACUGCC CCAGGAGUAC AUCCGCUGGG GCGAUGCCUG
 GGCUGUGGGA CCUGUCACCA UCGCCUGCCU CGGUGCCCUG GCCACCCUCU
 40 UUGUGCUGGG UGUCUUUGUG CGGCACAAUG CCACACCAGU GGUCAAGGCC
 UCAGGUCGGG AGCUCUGCUA CAUCCUGCUG GGUGGUGUCU UCCUCUGCUA
 45 CUGCAUGACC UUCAUCUUA UUGCCAAGCC AUCCACGGGA GUGUGUGCCU
 UACGGCGUCU UGGGGUGGGC ACUGCCUUCU CUGUCUGCUA CUCAGCCCUG
 CUCACCAAGA CCAACCGCAU UGCACGCAUC UUCGGUGGGG CCCGGGAGGG
 50 UGCCCAGCGG CCACGCUUA UCAGUCCUGC CUCACAGGUG GCCAUCUGCC
 UGGCACUUAU CUCGGGCCAG CUGCUCUUCG UGUUGCCUG GCUGGUGGUG
 55 GAGGCACCGG GCACAGGCAA GGAGACAGCC CCCGAACGGC GGGAGGUGGU

GACACUGCGC UGCAACCACC GCGAUGCAAG UAUGUUGGGC UCGCUGGCCU
 ACAAUGUGCU CCUCAUCGCG CUCUGCACGC UUAUGCCUU CAAGACUCGC
 AAGUGCCCCG AAAACUUCAA CGAGGCCAAG UUCAUUGGCU UCACCAUGUA
 CACCACCUGC AUCAUCUGGC UGGCAUUCUU GCCCAUCUUC UAUGUCACCU
 CCAGUGACUA CCGGGUACAG ACCACCACCA UGUGCGUGUC AGUCAGCCUC
 AGCGGCUCCG UGGUGCUUGG CUGCCUCUUU GCGCCCAAGC UGCACAUCAU
 CCUCUCCAG CCGCAGAAGA ACGUGGUUAG CCACCGGGCA CCCACCAGCC
 GCUUUGGCAG UGCUGCUGCC AGGGCCAGCU CCAGCCUUGG CCAAGGGUCU
 GGCUCCCAGU UUGUCCCCAC UGUUUGCAAU GGCCGUGAGG UGGUGGACUC
 GACAACGUCA UCGCUUUGA

identified herein as SEQ ID NO:3;

- (c) a nucleic acid compound complementary to (a) or (b); and
 - (d) a fragment of (a), (b), or (c) that are at least 18 bases in length and which will selectively hybridize to human genomic DNA encoding a human metabotropic glutamate receptor.
4. An expression vector capable of producing a human metabotropic glutamate receptor or a fragment thereof in a host cell which comprises a nucleic acid compound as claimed in **Claim 3** in combination with regulatory elements necessary for expression of the nucleic acid compound in the host cell operably linked thereto.
 5. A transfected host cell harboring an expression vector as claimed in **Claim 4**.
 6. A method of evaluating the effectiveness of a test compound for the treatment or prevention of a condition associated with a deficiency of stimulation of a human mGluR2 receptor which method comprises:
 - a) introducing into a mammalian host cell an expression vector comprising DNA encoding a human mGluR2 receptor as claimed in either one of **Claims 2 and 3**;
 - b) culturing said host cell under conditions such that the human mGluR2 receptor is expressed;
 - c) exposing said host cell expressing the human mGluR2 receptor to a test compound; and
 - d) measuring the change in a physiological condition known to be influenced by the binding of native ligand to the human mGluR2 receptor relative to a control in which the transfected host cell is exposed to native ligand.
 7. A method of evaluating the effectiveness of a test compound for the treatment or prevention of a condition associated with an excess of stimulation of a human mGluR2 receptor compounds which method comprises:
 - a) introducing into a mammalian host cell an expression vector comprising DNA encoding a human mGluR2 receptor as claimed in either one of **Claims 2 and 3**;
 - b) culturing said host cell under conditions such that the human mGluR2 receptor is expressed;
 - c) exposing said host cell expressing the human mGluR2 receptor to a test compound;
 - d) exposing said host cell expressing the mGluR2 receptor to glutamate simultaneously with or following the exposure to the test compound; and

e) measuring the change in a physiological condition known to be influenced by the binding of glutamate to the human mGluR2 receptor relative to a control in which the transfected host cell is exposed to only glutamate.

8. A method of evaluating the effectiveness of a test compound for use in the treatment or prevention of conditions associated with an excess or deficiency of stimulation of a human mGluR2 receptor comprising the steps of:

a) isolating a human mGluR2 receptor as claimed in **Claim 1**;

b) exposing said isolated human mGluR2 receptor to the test compound;

c) exposing the isolated human mGluR2 receptor to glutamate simultaneously with or following the introduction of the test compound;

d) removing non-specifically bound glutamate or test compound;

e) quantifying the concentration of test compound or glutamate bound to the human mGluR2 receptor; and

f) comparing the concentration of test compound or glutamate bound to the human mGluR2 receptor to a control in which no test compound were added.

(19)



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11)

EP 0 711 832 A3

(12)

EUROPEAN PATENT APPLICATION

(88) Date of publication A3:
27.08.1997 Bulletin 1997/35

(51) Int Cl.⁶: **C12N 15/12, C07K 14/705,
A61K 38/17**

(43) Date of publication A2:
15.05.1996 Bulletin 1996/20

(21) Application number: **95308033.0**

(22) Date of filing: **09.11.1995**

(84) Designated Contracting States:
**AT BE CH DE DK ES FR GB GR IE IT LI LU NL PT
SE**

(30) Priority: **14.11.1994 US 337797**

(71) Applicant: **ELI LILLY AND COMPANY**
Indianapolis, Indiana 46285 (US)

(72) Inventors:
• **Burnett, James Paul, Jr.**
Indianapolis, Indiana 46250 (US)

- **Mayne, Nancy Gail**
Indianapolis, Indiana 46226 (US)
- **Sharp, Robert Leon**
Indianapolis, Indiana 46237 (US)
- **Snyder, Yvonne Marie**
Indianapolis, Indiana 46256 (US)

(74) Representative: **Hudson, Christopher Mark**
Lilly Industries Limited
European Patent Operations
Erl Wood Manor
Windlesham Surrey GU20 6PH (GB)

(54) **Human metabotropic glutamate receptor, m Glu R2**

(57) This invention describes a novel human glutamate receptors, designated mGluR2. This invention al-

so encompasses nucleic acids encoding this receptor, or a fragment thereof, as well as methods employing this receptor and the nucleic acid compounds.

EP 0 711 832 A3



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 95 30 8033

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
E	WO 96 06167 A (CIBA GEIGY AG ; FLOR PETER JOSEF (DE); KUHN RAINER (DE); LINDAUER K) 29 February 1996 * the whole document *	1-8	C12N15/12 C07K14/705 A61K38/17
X	NEURON, JAN 1992, 8 (1) P169-79, UNITED STATES, XP000674842 TANABE Y ET AL: "A family of metabotropic glutamate receptors."	1-5	
Y	* the whole document *	6-8	
Y	EP 0 569 240 A (LILLY CO ELI) 10 November 1993 * page 1, line 1 - page 3, line 26; claims 1-10; examples 4,6 *	6-8	
P,X	EUR J NEUROSCI, APR 1 1995, 7 (4) P622-9, ENGLAND, XP000674823 FLOR PJ ET AL: "Molecular cloning, functional expression and pharmacological characterization of the human metabotropic glutamate receptor type 2."	1-5	
	* the whole document *		
A	MOL PHARMACOL, APR 1994, 45 (4) P626-36, UNITED STATES, XP000674841 CATANIA MV ET AL: "Metabotropic glutamate receptor heterogeneity in rat brain."		
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 2 June 1997	Examiner Nauche, S
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

EPO FORM 150 (3.92) (P/0401)